A COMPARATIVE STUDY OF ADRENAL MEDULLARY AND CARDIOVASCULAR RESPONSES TO HAEMORRHAGE.

by

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THESIS

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I certify that the work presented in this Thesis is my own.

Abdulmuttalib Yousif Yakob Alabood.

To my Parents, Uncles and Sisters.

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INTRODUCTION

Haemorrhage is a condition characterized by a lowering of the circulating blood volume leading to a reduction of cardiac output, fall in arterial blood pressure and lowered overall perfusion of the tissues.

The cardiovascular compensating responses to haemorrhage in man and many mammals have been extensively documented. The immediate changes involve an increase in heart rate (noted as early as 1733 by Stephen Hales), a rise in total peripheral resistance and transfer of tissue fluid into the circulation. These changes occur, directly or indirectly from the reflex activation of the sympathetic nervous system, due to reduced arterial baroceptor and cardiac receptor activity (Korner, 1971) and, except in the rabbit, an increase in arterial chemoceptor discharge (Landgren and Neil, 1951; Paintal and Riley, 1966). The cardiac tachycardia is due to increased sympathetic and decreased vagal efferent activity. Strong vasoconstriction occurs in the circulations of the kidney, the digestive tract and skeletal muscle (Greenway and Lawson, 1966). The wide extent of the role of the sympathetic nervous system in haemorrhage has been the subject of an extensive review by Chien (1967).

There is considerable evidence for a rise in adrenal medullary catecholamine secretion in most species studied during haemorrhagic hypotension. There is also evidence of a preferential secretion of adrenaline over noradrenaline

during haemorrhage. Much of this work is reviewed by Malmejac (1964). However, in spite of the considerable body of literature on the subject little is really known about the significance of the catecholamine release in this particular stress situation. Adrenaline is now widely regarded as having its most important effects on metabolic rather than cardiovascular responses. In this context the rabbit is of particular interest since its adrenal medulla secretes virtually no noradrenaline the output being entirely, or almost entirely adrenaline. The dog, by comparison, secretes a much greater proportion of noradrenaline (20 - 40% of total catecholamine) (Eränkö, 1955). Evidence that, in the rabbit, adrenaline produces purely vasoconstrictor responses in the skinned hind limb (Powis, 1974) might indicate a more important role for this substance in cardiovascular compensatory reactions in this species - this investigation was undertaken, therefore, to study the release of adrenal catecholamines in the rabbit during haemorrhagic hypotension and to compare it with reactions occuring in the dog.

Another aspect of the investigation was associated with the possibility that in haemorrhage adrenal catecholamines might be released other than by sympathetic nerve stimulation. In particular, its release by angiotensin has been studied. It has been shown that plasma angiotensin is raised during haemorrhage in the dog (Hodge, Lowe and Vane, 1966) and the cat (Hall and Hodge, 1971) and that the rise in angiotensin levels

precedes that of the catecholamines (Hall and Hodge, 1971). The ability to release adrenal catecholamines has been reported by several groups of investigators (Feldberg and Lewis, 1964; Staszewska-Barczak and Vane, 1965, 1967; Peach, Cline and Watts, 1966). It was of particular interest in this investigation to examine the possible role of angiotensin in the release of adrenal catecholamine in the dog and rabbit during haemorrhagic hypotension.

REVIEW OF PREVIOUS LITERATURE

METHODS

1. Chemical methods for the estimation of Catecholamines.

The methods depend on the production of a colour reaction or fluorescence by adrenaline and noradrenaline under certain conditions.

Colorimetric methods.

Various colorimetric methods for estimating adrenaline exist which are described by Barker, Estland and Evers (1932) under three main groupings for the purpose of this survey:-

A. Those depending primarily on the formation of a red oxidation product; including the following oxidizing reagents, ferric chloride, potassium iodate, mercuric chloride, and many others including hydrogen peroxide, potassium ferricyanide, permanganate and dichromate, sodium bismuthate, sodium hypobromite, manganase dioxide and the halogens.

B. Those depending on the presence of the catechol grouping, including the ammonium molybdate test which give a yellow colour, and Folin's test which gives a deep blue colour.

C. Miscellaneous tests including diazobenzene sulphonic acid and the ninhydrin reactions.

Another reaction, uses ferrous sulphate followed by sufficient Disodium phosphate to bring the mixture to neutrality. A blue colour appears which is fairly stable

but rapidly fades through a purple colour if too much alkali is added.

All the above reactions did not seem sensitive enough to estimate traces (less than 1 part in 10,000) of adrenaline. Some of these tests are unsatisfactory even from the qualitative point of view; others are non specific, the latter criticism applying to the ferric chloride, ammonium molybdate and phosphotungstic acid reactions, although the latter is extremely sensitive, and gives good results with comparatively pure adrenaline solution.

Comparing those tests with the biological methods showed that neither phosphotungstic acid test nor the molybdate test gives reasonably accurate results with gland extract. The potassium persulphate reaction was investigated more thoroughly (Barker, Eastland and Evers, 1932) and gives results comparable with those obtained by the biological methods when the necessary conditions which include control of temperature and pH are observed. Sensitivity is sufficient for the determination of adrenaline in the suprarenal glands themselves.

Other methods described were by Viale (1933); Kobayashi (1935) and Whitehorn (1935) but may only be applied where the concentration is more than 1 g/ml. They are useful for estimations of adrenaline in suprarenal extract but not in other tissues. A modification of Whitehorn's method was introduced by Shaw (1938) which is simpler and more sensitive. The method depends on the fact that adrenaline reduced arsenomolybdic acid

with the formation of a blue colour. It has been found that brief preliminary treatment of adrenaline with alkali in the presence of oxygen increases the colour considerably. When this test is applied to the suprarenal extract it gives results agreeing with biological estimations. Adrenaline can be estimated in tissues using this method, provided that the concentration exceeds 10^{-7} grams. If the concentration is lower, results must be interpreted with caution.

Fluorimetric Methods.

These methods depend on the finding that adrenaline in alkaline solution and in the presence of oxygen becomes fluorescent. A sensitive method for adrenaline described by Gaddum and Schild (1934) showed that if 0·1 cc of 6N NaOH was added to 1 cc of adrenaline solution, a bright apple-green fluorescence appeared which lasted for about 1 minute with low concentration and for several minutes with higher concentrations. When they attempted to apply this test to blood serum, it was found that the fluorescence due to low concentration of added adrenaline was obscured by a blue fluorescence due to other substances in the blood. They noted that in identical experimental conditions the fluorescence caused by noradrenaline is much weaker than that produced by adrenaline and accordingly many workers tried to apply the method differentially to estimate adrenaline and noradrenaline. All these attempts were difficult

to evaluate because of the instability of the fluorescent substances and the varying experimental conditions employed. An important advance occurred when adrenaline and noradrenaline contained in purified plasma were oxidized by potassium ferricyanide at pH 6·0 (Eade and Wood, 1958; Miller and Benfey, 1958) and the fluorescent intensity, developed after treatment with sodium hydroxide and ascorbic acid was measured at 365 m and 436 m using a fluorescence attachment to a spectrophotometer.

The chemical determination of the small amounts of adrenaline and noradrenaline in plasma seemed to be possible by fluorimetric measurement, following separation of the catecholamines from the plasma by aluminium oxide or ion exchange column. The methods were based on two principal chemical reactions, the trihydroxyindole reaction (Lund, 1949a, 1949b) and the ethylene-diamine condensation reaction (Weil-Malherbe and Bone, 1952), the latter reaction appearing to be less specific than the former.

In the trihydroxyindole methods adrenaline and noradrenaline were oxidized to adrenochrome and noradrenochrome, which in alkali were reduced to the trihydroxyindoles, adrenolutine and noradrenolutine, respectively, which are strongly fluorescent. The fluorescence of the lutines was stabilized by the addition of ascorbic acid and measured at 545 and 535 m respectively following activation at 425 and 410 m.

In 1955, Eränco studied the distribution of adrenaline and noradrenaline in the adrenal medulla using the trihydroxy reaction. Euler and Floding (1955) used a trihydroxyindole micro method for differential estimation of adrenaline and noradrenaline.

In 1952, Weil-Malherbe and Bone used a method based on the ethylene diamine condensation reaction for the assay of catecholamines. The method consists of the following steps (a) filtration of the plasma-buffer mixture (pH 8·4) through a column of acid-washed alumina and elution of the absorbed amines by dilute acetic acid; (b) heating of the eluate at 50° with a mixture of ethylene diamine and ethylene diamine dihydrochloride; (c) extraction of stable fluorescent condensation product with isobutanol; (d) measurement of fluorescence.

Goldenberg, Serlin, Edwards and Rapport (1954) introduced a short concentration procedure consisting of absorption of catecholamines in hydrolyzed urine on to an aluminium oxide column from which they were subsequently eluted. The assay of adrenaline and noradrenaline in adrenal glands was carried out by separation of the two amines using paper chromatography and estimating each separately by fluorimetry after elution from the paper (Eade and Wood, 1958).

A modification of the method of Euler and Floding for differential fluorimetric estimation of adrenaline and noradrenaline has been described by De Schaepdryver (1958) which could be used for testing of urine and plasma

The sensitivity of the method using 20 ml blood samples, appeared sufficient to permit the accurate estimation of adrenaline and noradrenaline concentrations in plasma in the order of 3×10^{-3} and 5×10^{-3} g/l respectively (Miller and Benfey, 1958). The addition of ethylendiamine to the ascorbic acid (Euler and Lishajko, 1961) improved the stability of both sample and blank.

The paper of Auton and Sayre (1962) is important as it gives a detailed survey and critical analysis of the many variants of the trihydroxyindole method and column separation techniques in use up to that date. Their method forms the basis of almost all methods subsequently introduced. Other modifications to the trihydroxyindole method (Haggendal, 1963a) led to an appreciable improvement of the sensitivity obtained. The blank values were considerably reduced and stabilized by substituting dimercaptopropanol (BAL) in sodium sulfite solution for ascorbic acid. The eluate values were reduced and the degree of purification increased by a modified ion exchange procedure (Dowex 50W - X8). When this procedure was applied to 10 ml plasma obtained from normal human subjects at rest, noradrenaline spectra with two activation peaks were usually obtained. In 1970, Berzini, Brunori and Volori, described a sensitive and specific method to determine adrenaline and noradrenaline in human plasma, a blood sample of 10 - 20 ml was needed.

O'Halon (1970) described a fluorimetric method for

the estimation of subnanogram concentrations of adrenaline and noradrenaline. Allison and Powis (1971) used trihydroxyindole method to estimate catecholamine secretion during stimulation of the nasal membrane in the rabbit. The important feature of their technique was the small blood samples (0·12 ml).

Radioenzyme Assay Methods.

DaPrada and Zürcher (1976); Bühler, DaPrada and Haefely (1978) used a highly sensitive radioenzymatic method for catecholamine determination in plasma samples of man and various animal species. The catecholamines, in this method, were converted to their O-methylated labelled derivatives by catechol-O-methyl-transferase in the presence of tritiated-S-adenosyl-methionine. The radioactive methylated derivatives were then extracted and separated by thin-layer chromatography. (^{3}H)-methoxy-tyramine was scraped off and assayed directly for its radioactivity. The tritiated metanephrine and normeta-nephrine were first converted to vanilline by periodate cleavage, and thereafter extracted into toluene and their radioactivity measured using a liquid scintillation spectrometer.

2 - Bioassay Methods for Estimating Catecholamine.

Many investigators have shown the value of biological techniques in the estimation of catecholamines. One of the earliest methods for assaying the secretion of the suprarenal gland was that by Elliott (1912) modified by Bain and Suffolk (1936) and Bain, Gaunt and Suffolk (1939). This method was based on the rise of blood pressure caused by adrenaline, in a cat, with the spinal cord destroyed to the fourth cervical segment in order to avoid vasomotor reflexes. Euler (1948) treated the cat with 1 mg/kg atropine sulphate and 3 mg/kg hexamethonium bromide to reduce reflex vasodilator responses and bradycardia. It was found that hexamethonium by lowering the blood pressure of the cat to about 60 mmHg provided a much more sensitive preparation for assaying pressor amines.

For the diagnosis of cases of pheochromocytoma Euler,(1951) and Goldenberg and co-workers,(1954) absorbed the urinary catecholamines on precipitated aluminium hydroxide, followed by elution, desalting and concentration in vacuo. The extracts were studied using the cats blood pressure response accompanied by other chemical methods. A rat preparation may also be used in the same manner (Landgrebe, Macaulay and Waring, 1946). This method is reported to be more sensitive to noradrenaline than adrenaline. Shipley and Tilden in 1947 described a pithed rat preparation useful for assaying

pressor substances. After the rat was anaesthetised with sodium amytal, atropine sulphate was given. A rod was then inserted obliquely into and through the eye socket at the angle of approximately 45° to the long axis of the rat, the rod passing down the whole length of the spinal canal and was left in place for the whole of the experiment. Blood pressure fell sharply and respiration was maintained with a pump. Crowford and Outschoorn (1951) modified the method of Laudgrebe et. al., (1946), a rat 200 - 250 g body weight was anaesthetized with urothane subcutaneously, and the trachea cannulated. Blood pressure was measured from one common carotid artery while the other carotid was ligated. Both vagi and femoral nerves lateral to venous cannulae were cut. The animal was then left for 15 - 20 minutes until the blood pressure settled to a steady level before commensing the assay.

Rats treated with 100 μg/100 g atropine sulphate and 1 mg/100 g hexamethonium bromide were used by several workers, (Outschoorn, 1952; Vogt, 1952; Silver, 1960; and Comline and Silver, 1966). Blood pressure was lowered to about 50 mmHg which decreased the effect of depressor substances in the injected plasma samples. The rat could be used immediately for assay, a considerable advantage over the earlier methods. After 45 - 60 minutes the blood pressure was again lowered by a further injection of hexamethonium in double the previous dose. The workers suggest that this technique

when applied to rats gives a sensitive preparation for estimating both adrenaline and noradrenaline (Holzbauer and Vogt, 1956). Noradrenaline is three to four times more effective than adrenaline in its effect in raising blood pressure of a pithed rat preparation (Muscholl and Vogt, 1957) which precluded its use in the current work in the rabbit whose adrenal glands probably release almost entirely adrenaline alone. Furthermore, interference of other substances which might be generated during haemorrhage, e.g. angiotensin, causes a pressor effect on pithed rat preparation (Shipley and Tilden, 1947). This made it unsuitable for this investigation.
Gaddum and Goodwine (1947) and Peart (1949) used the isotonic contraction of the nictitating membrane to assay catecholamines. In some cases the membrane was denervated by removal of the superior cervical ganglion in a preliminary aseptic operation under general anaesthesia 7 - 10 days before the main experiment. Adrenaline dilated the pupil while noradrenaline caused contraction of the nictitating membrane. Stimulation of the splenic nerves caused the appearance in the plasma of pharmacologically active substances, which leads to the contraction of the nictitating membrane. This work was based on the earlier investigations of Cannon and Rosenblueth (1937) and Isola and Bacq (1946).
Some care in the interpretation of results is required. The contraction of the denervated nictitating membrane is more sensitive to

noradrenaline than adrenaline and these factors make it unsuitable for the current experiments, apart from the obviously expensive nature of the preparation.

Some workers have used the vasoconstriction of the perfused rabbit ear by catecholamines as a test tissue (Gaddum and Kwiatkowski, 1939; Fleckenstein, 1952; Powis, 1973), when the ear itself was used as a detector, perfusion pressure was kept constant. Active solutions were injected into the special cannula, and changes of outflow recorded. Adrenaline caused vasoconstriction in concentrations of 10^{-8} g/l and over. Gaddum in his review in 1953 on his assay procedure mentioned that Schlossmann (1927) studied various methods of estimating adrenaline and recommended the use of the rabbit ear perfused at constant pressure with a solution containing serum and citrate. The decrease in blood flow in the hind limb vascular beds of an anaesthetized rabbit in response to catecholamines (Powis, 1974) is due to vasoconstriction and has been used as an index of circulating levels of the amines.

Smooth muscles of various tissues have been used for the assay of catecholamine. Probably the work of Loewi (1921) was one of the basic pieces of work in biological assay technique. He showed that stimulation of the adrenergic nerves in frogs heart liberate a fluid which when perfused into a second heart caused its stimulation and when injected in frog's arteries caused constriction (Brankman and Van Dam, 1922). The frog's

heart was isolated and perfused with Locke's solution diluted to 1·4 times its volume with water (Gaddum and Kwaitkowski, 1939). This preparation usually reacted with an increase in the size of the beat using the myocardiograph recorder (Cushny, 1910) with concentrations of 10^{-9} g/l of adrenaline, and sometimes to concentrations of 10^{-10} g/l. An accurate method of assay using the perfused frog's heart has been described by West (1943). Having the heart free from the pericardium and having ligated the anterior venae cavae, a venous cannula full of Ringer's solution, was inserted into the posterior vena cava. The heart was then removed from the animal and the movement recorded isotonically. Repeated infusion of the same dose of standard adrenaline produced similar responses, but after 4 - 5 hours the response diminished gradually. Winter male frogs provided the best test objects.

Von Euler (1934) used the inhibitory effect of catecholamine on the isolated rabbit jejunum for the estimation of adrenaline. The isolated chick rectum has also been used (Barsaum and Gaddum, 1935; Gaddum and Kwiatkowski, 1939; van Euler, 1951; Gaddum, 1953; and Galviano, Bass and Nykiel, 1960) for the assay of adrenaline and noradrenaline.

The contraction of the isolated uterus of young rats (4 - 5 weeks) induced by acetylcholine injections made at set time intervals (2 mins) was inhibited by adrenaline (Jalon, Bays and Jalon, 1945). Both horns

of the uterus were tied together side by side and the response to adrenaline depended on the difference between the effect produced under its influence and the effect that was produced by acetylcholine alone.

Gaddum and Lembeck (1949); Gaddum, Peart and Vogt (1949) and Peart (1949) used a rat uterus and colon preparation suspended in a solution of the following composition (g/l) NaCl 9, KCl 0·42, $CaCl_2$ 0·06, $NaHCO_3$ 0·2, glucose 0·5 at 30°C. Contraction was produced every 2 minutes by acetylcholine or by carbachol, and the assay depends on the inhibition of these contractions by adrenaline or noradrenaline. The uterus is more sensitive to adrenaline and the colon to noradrenaline so that the contractions of both in a mixture could be roughly determined by parallel quantitative assay on these tissues. The maximum sensitivity of this method is in the range of 1 μg adrenaline/l of blood, being about 100 times more sensitive to adrenaline than to noradrenaline. Isolated uterus of a non-pregnant rat in dioestrus was used by Peart (1949) and Mann and West (1950), and the preparation was sensitive to 10^{-10} g/l adrenaline or 5×10^{-9} g/l noradrenaline in tyrode solution at 37°C. Watts (1956) also used the rat uterus to estimate adrenaline levels during haemorrhage in the dog. The blood adrenaline level reached a maximum of 37·2 μg/l after a haemorrhage of 40 ml/kg. The sensitivity of this method was in the range of 1 μg adrenaline/l of blood.

A strip of tissue from the rat stomach has been

reported to be contracted by 5-Hydroxytryptamine (Vane, 1957) and when catecholamines were added the size of contraction was reduced. He suggested, that if the tone of the preparation could be raised it would become more sensitive to the relaxant action of catecholamines. This observation gave rise to the use of a strip of fundus from rat's stomach suspended in Krebs solution containing 5-HT (Armitage and Vane, 1964) for the rough estimation of a catecholamine mixture by recording the relaxation of the strip. When isoprenaline, adrenaline or noradrenaline was added to the bath in a concentration of 0·2 - 2 ng/ml the preparation was most sensitive to isoprenaline and least sensitive to noradrenaline. When the strip is superfused the responses to catecholamines were greater than when the tissue was totally immersed in Krebs solution.

Regoli and Vane (1964a) tested many substances on various preparations of isolated smooth muscle and they observed that rat stomach strip was relaxed by adrenaline and noradrenaline while it was contracted by angiotensin, bradykinin and acetylcholine. Histamine seemed not to affect the preparation. The chick rectum is another tissue widely used for assaying catecholamine. It is 20 - 100 times more sensitive to adrenaline than to noradrenaline and is therefore confined to assays where adrenaline only is involved (Mann and West, 1950).

However, the rat stomach strip appeared to be the most sensitive tissue for detecting circulating levels

of catecholamine (Vane, 1964) and it gives an estimate of total amines, for its sensitivity to both adrenaline and noradrenaline was of the same order (adrenaline twice as active as noradrenaline).

In 1958 Vane used the blood bathed isolated organ technique to test the circulating blood for a variety of active substances and later he described a method for the assay of many hormones with circulatory effects after their injection or release. The method could be used for cats, dogs and rabbits, and consisted of bathing or superfusing an isolated smooth muscle preparation in a stream of heparinised arterial blood taken from and returned to the animal at a constant rate (Vane, 1964). The tone of the smooth muscle was affected by a small change in the concentration of various amines and after testing both rat stomach strip and chick rectum, Vane suggested that the stomach strip was the most sensitive tissue for the detection of circulating catecholamines. A rat stomach strip together with chick's rectum continuously superfused with arterial blood was used to estimate the catecholamines released from the adrenal medulla of the dog and cat (Staszewska-Barczak and Vane, 1965, 1967) and Guinea pigs (Piper, Collier and Vane, 1967; Piper and Vane, 1967) by injection of peptides into the circulation.

In the second Gaddum Memorial Lecture (Vane, 1969) described in detail the use of blood bathed organ techniques to study the release and fate of several vaso-active

hormones in the circulation at one time and he concluded that adrenaline was one of the circulating hormones which passed through the lung unchanged and was removed while passing through the peripheral vascular beds. He also showed that when the stomach strip was first superfused with blood there is often a change in resting tone. This contraction, which is much stronger in the rat stomach strip than in any other assay tissues may be a reaction to an unknown substance in the blood or to change in oxygen tension or ionic composition. After 20 - 60 minutes of superfusion the tissue reached a stable base line.

Since then many workers have used the rat stomach strip to test the blood for the presence of catecholamine released by angiotensin in Guinea pig (Piper and Vane, 1967) and cat and dog (Marley, 1961; Staszewska-Barczak and Vane, 1965, 1967).

For the estimation of urinary catechol amines (Helmer, 1975) and blood catecholamines (Douglas and Rubin, 1961a,b and 1963) a spirally cut strip of rabbit thoracic aorta, mounted in a muscle bath filled with oxygenated Krebs solution has been used. The movement of the strip was recorded by isotonic lever. Addition of catecholamines to the bath caused contraction of the strip. In the same way Fowler, Shabetai and Holmes (1961) used rabbit aortic strip to estimate adrenal medullary secretion during haemorrhage. Plasma samples were used for the measurement and corrected to whole blood from the haematocrit readings. Changes in the catecholamines and

angiotensin level in the cats and dogs during haemorrhage were measured (Hall and Hodge, 1971) by rat stomach strip and rat colon successively using the continuous superfusion technique of Vane (1964) by establishing an extra corporeal circuit needing only 10 - 15 ml blood. The great advantage of this method is that no blood samples were required to be removed, in contrast to the chemical methods most of which require 10 - 20 ml blood for each estimation. Using these tissues, the superfusion technique offered a means of continuously assaying changes in the circulating levels of catecholamines. The sensitivity of the assay method was shown to compare favourably with chemical methods, being able to detect changes in levels of the order 0·5 - 1.0 ng/ml at its best.

3 - Chemical Assay of Renin and Angiotensin.

Biochemical methods have also been used for the estimation of renin in human and dog plasma (Brown, Davies, Lever, Robertson and Tree, 1964) and rabbit (Lever, Robertson and Tree, 1964). Clinically measurements have been made to estimate the changes in renin concentration in the plasma of man after haemorrhage (Brown, Davies, Lever, Robertson and Veriory, 1966). The technique consists of an estimation of renin concentration by determining the initial velocity of angiotensin formation under standard conditions of incubation with a substrate. Angiotensinase-free ox-serum substrate

is used. The recoveries are consistent and the method sufficiently sensitive to measure peripheral venous plasma renin in both normal conditions and conditions associated with depression of plasma renin concentration.

An improved assay method for measuring renin concentration and activity has been described by Skinner (1967) based on the denaturation of renin substrate in which separation and concentration steps are avoided and the recovery of renin is complete. In this plasma renin activity method effective inhibition of angiotensinase is achieved by warming plasma at pH 4·5 with EDTA followed by dialysis at pH 7·5. Neither renin nor renin substrate is affected by this treatment. In plasma renin concentration methods, renin substrate is selectively denatured by warming at pH 3·3 followed by dialysis at pH 7·5 and addition of a standard substrate prepared from nephrectomized sheep. Incubation results in a linear increase of pressor material which is assayed without extraction on rat blood pressure against synthetic angiotensin.

Ryan, Mckenzie and Lee (1968) described a rapid simple method for the assay of renin in rabbit plasma. EDTA, 2,3-diamercaptopropan-1-Ol and chlorhexidine gluconate cause complete inactivation of plasma enzymes that degrade angiotensin I, but have no effect on the reaction of renin with its substrate. Thus it is possible to measure renin in plasma by its ability to catalyze the release of angiotensin. But the method of purification of plasma angiotensin is a difficult one to apply to the

present study especially for the rabbit as many blood samples are required. This itself might cause increase in level of angiotensin in the circulating blood.

4 - Bioassay Methods for Renin and Angiotensin.

The intravenous injection of extracts prepared from fresh kidney of several species have been shown (Pickering and Prinzmetal, 1938) to produce prolonged rise of blood pressure in the unanaesthetized rabbit and pithed rat preparation.

Shipley and Tilden (1947) have shown the latter preparation to be useful for the estimation of angiotensin which causes a rise in blood pressure related to concentration.

Another bioassay procedure used for the determination of renin (Hass and Goldblatt, 1959; Scornik and Paladini, 1961) is based on the elevation of mean arterial blood pressure of the normal, unanaesthetized dog as a result of intravenous injection of renin, but the pressure effects of angiotensin could interfere with other substances in the blood such as catecholamine (Shipley and Tilden, 1947) which reduce its uses as an assay technique.

The constrictor effect of angiotensin on smooth muscle preparations has been used for many years as the basis of many bioassay techniques. Tissue used includes the rabbit intestine (Page, 1940) and the Guinea pig ileum (Collins, 1948). The latter is the most sensitive but is not specific. In order to find

a sensitive and specific replacement for the rat uterus Regoli and Vane (1964a) have compared the responses of different parts of the intestine of the rat, Guinea pig, chicken and pigeon to synthetic angiotensin and reported that the rat ascending colon produced the best results. While they preferred the use of the superfusion technique they showed two major disadvantages. The first was the high spontaneous activity of the tissue and the second was that catecholamines reduced the response to released angiotensin. The addition of pronethalol to the bathing solution relaxed the colon strip, reduced its spontaneous activity, and completely abolished the interference of catecholamines and diminished that of bradykinin.

Since then the rat ascending colon has been used for continuous estimation of angiotensin formed in the circulation (Regoli and Vane, 1966) of the dog and during haemorrhage in the cat and dog (Hall and Hodge, 1971). Robertson and Rubin (1958) showed an indirect action of angiotensin I on smooth muscle. It appears that at least part of the action of angiotensin I on some isolated ileal preparations is indirect, being mediated through cholinergic neurones.

The Circulatory and associated changes in Haemorrhage.

Haemorrhage, or hypovolaemia leads to a decreased venous return, cardiac output and arterial blood pressure. The early decrease in arterial blood pressure is sensed by the baroceptors and the fall is offset or reversed by sympathetic nervous effectors causing vasoconstriction and increased rate and strength of myocardial contraction. A sustained reduction in blood pressure leads to a lowered capillary pressure which permits an influx of interstitial fluid into the vascular system to augment circulating plasma volume. Later, reduction of oxygen carrying capacity and increased hydrogen ion concentration of the blood will lead to a chemoreceptor stimulation and further sympathetic activation.

In addition sympathetic stimulation of the spleen results in its contraction with ejection of blood of high haematocrit into the depleted circulation. The combined effect of these erythrocytes plus the increased plasma volume often results in little change in the packed cell volume of the circulating blood in the short term. Wendall Nelson (1976) claims that by these two mechanisms the dog can replace up to 20 percent of its circulating blood volume.

Peripheral vasoconstriction affects terminal arterioles, pre-capillary sphincters and venules and results in reduced perfusion and varying degrees of tissue ischemia. This stimulation persists until local hypoxia leads to a loss of blood vessel muscle tone

and the pooling of blood in the capillaries of many vascular beds.

Tissue hypoxia resulting from the ischemia leads to a decreased oxidative function in cells with a general reduction in metabolic activity. Glucagon and catecholamines are secreted leading to glycogenolysis and hyperglycaemia. The action of insulin is antagonised by the released catecholamines and cortisol. Glucose metabolism stops at the pyruvate-lactic acid stage resulting in a marked increase in hydrogen ion and acidaemia.

Liver and kidney functions are depressed as indicated by increased levels of plasma ammonia and urea nitrogen. Decreased urine production, as a result of the hypotension and vasoconstriction is followed by complete renal shut down when arterial blood pressure falls below about 70 mmHg. In addition, the fall of renal blood pressure stimulates the secretion of renin by the juxtaglomerular cells. This enzyme acts on the α - 2-globulin fraction of the plasma protein to release a short peptide fragment which is converted into a powerfull vasoconstrictor, angiotensin II. This also stimulates aldosterone secretion which promotes sodium and water retention.

All these responses are essentially short term compensations. If perpetuated over longer periods they invariably lead to the onset of shock and death unless fluid replacement is carried out.

The Circulatory Changes in Haemorrhage.

It is well known that haemorrhage involves a loss of blood resulting in a decreased blood volume and having as its main immediate result a decreased cardiac output (Cournand, Riley, Bradley, Breed, Noble, Lauson, Gregerson and Richards, 1943). In the intact anaesthetized dog a haemorrhage amounting to about 2·1 per cent of body weight was found by Meek and Eyster (1921) to be necessary before the diastolic heart size, and presumably output were reduced. The cardiac output was initially reduced during haemorrhage in unanaesthetized rabbits (Chalmers, Korner and White, 1967) but later returned to about normal levels. In the anaesthetized dog, the minute volume output is maintained, with the exception of slight drop immediately after bleeding, mean arterial blood pressure also being reduced (Cannon, 1923; Cournand, Riley, Bradley, Breed, Noble, Lauson, Gregerson and Richards, 1943; Remington, Hamilton, Caddell, Boyd and Hamilton, 1950b; Korner, 1971). The arterial blood pressure was reduced in dogs with or without the sympathetic nervous system inactivated by surgical complete sympathectomy (Chien, 1958). After haemorrhage central venous pressure was lowered in anaesthetized dogs (Remington, Hamilton, Caddell, Boyd and Hamilton, 1950a; Chien, 1958; Rothe and Selkurt, 1964) and rabbits (Chalmers, Korner and White, 1967).

Sympathetic Nervous Mechanisms in Haemorrhage.

Changes of Heart Rate.

Possibly one of the most important compensating responses to haemorrhage is the increase in heart rate caused by increasing sympathetic activity (Cannon, 1923; Walton, Richardson, Walton and Thompson, 1959; Saranoff and Mitchell, 1962). The increase in heart rate in anaesthetized dogs after haemorrhage (Chein, 1958) was abolished after sympathectomy. Increased heart rate was also observed during haemorrhage in unanaesthetized rabbits (Chalmers, Korner and White, 1967). In the de-efferented rabbits treated with guanethidine to block adrenergic nerve transmission there was no tachycardia instead bradycardia commenced about 10 - 15 minutes after the start of bleed.

Crowell and Guyton (1961, 1962) studied the evidence favouring a cardiac mechanism in irreversible haemorrhagic shock in the dog. It was found that no significant change in oxygen consumption, cardiac output or peripheral resistance during the transition phase during which the animal passed from the reversible stage of shock to an irreversible stage. The evidence is entirely consistent with the idea that the irreversible stage of haemorrhagic shock is caused by rapid progressive cardiac failure of unknown cause.

Changes in Total Peripheral Resistance.

A major compensating mechanism to haemorrhage is the rise in total peripheral resistance (Meek and Eyster, 1912; Cannon, 1923; Walton, Richardson, Walton and Thompson, 1959).

In 12 dogs subjected to haemorrhage (Cope, 1911) 9 showed an increase in total resistance following loss of blood, the other 3 showing a decrease. Of the 9 cases, 4 were followed by a compensatory rise of arterial pressure, but in 4 no such reaction followed. Of the 3 cases showing a decreased peripheral resistance after loss of blood, in only one was there a compensatory rise. He concluded the increased total peripheral resistance is not the only factor in the return of blood pressure.

Haemorrhage is accompanied by a reduction in the size of veins (Alexander, 1963; Green, Rapela and Conrad, 1963).

Nervous Control of Vasoconstriction.

In 1879, Mapother addressing the surgical society of Ireland suggested that the most marked physical change caused by shock was contraction of the arterioles. His conclusion was that this was due to shock paralysing the dilator nerves. It was, nevertheless, one of the earliest statements of the importance of peripheral vasoconstriction and rise in total peripheral resistance in haemorrhage. In 1905, Malcolm stated that the first effect of an injury is a constriction of the vessels,

a further indication of the importance of vasoconstriction in response to loss of blood.

When haemorrhage is performed in a dog, the fall of central blood pressure leads to enhanced cardiac activity and vasoconstriction of the blood vessels due to an automatic protective mechanism mediated through the carotid sinus baroceptors to maintain the blood supply to vital organs. This response is not evident when the animals pass into shock (Bartlett, 1912; Seelig and Joseph, 1916; Erlanger, Gesell and Gasser, 1919; Meek and Eyster, 1921).

Watts and Bragg (1957) have suggested that it is possible that the increased concentration of adrenaline in the circulation is at least partially responsible for the intense vasoconstriction and decreased peripheral blood flow in certain tissues in dogs. Powis (1974) showed that simultaneous stimulation of the sympathetic nerves to the hind limb of rabbit accompanied by infusion of adrenaline in quantities that could be liberated by splanchnic nerve stimulation at equivalent frequencies showed that the vasoconstrictor effect exerted by the individual components are additive. However, the effects produced directly by the sympathetic nerve supply to the blood vessels overshadow those produced by the circulating catecholamines. The results are discussed in the context of the possible vascular role of the adrenal medullary hormones in the rabbit. It would appear that in any physiological condition in which the frequency of

discharge in the splanchnic nerves and sympathetic vasoconstrictor nerves of the hind limb is the same, the vascular responses produced by the adrenal catecholamines would be negligible compared with those produced by the vasomotor nerves.

Vasoconstriction has a limited beneficial effect and prolonged vasoconstriction accelerates the development of circulatory collapse after haemorrhage. This area of study was given a new impetus by the findings of Wiggers, Ingerhan, Roemhild and Holdberg in 1948 that the sympathetic α - blocker, dibenamine, administered to dogs only 30 minutes before haemorrhage resulted in a greater proportion of survival animals to a standard bleed procedure (Remington, Hamilton, Boyd, Hamilton and Caddell, 1950b). In 1952, Brandfonbrener and Giller found that dibenamine did not prolong survival in haemorrhagic shock. Furthermore, they found no correlation between renal blood flow and survival rate.

A small elevation of venous pressure at constant flow causes constriction of all small vessels (Haddy and Scott, 1964). This constriction does not appear to be related to tissue pressure, oxygen, metabolites or reflexes other than those acting locally. Abel, Waldhausen and Selkurt (1965) suggested that the monkey may avoid splanchnic pooling during haemorragic shock by shunting blood from portal to systemic veins. Nevertheless, their animals all died by mechanisms which they could not fully explain. Possibilities included loss of intravascular

volume, cardiac failure, or central nervous system damage and loss of vasomotor reflex control.

After haemorrhage in cats, the flow from all vena caval segments decreased (Greenway and Lawson, 1966). Flow rates from different segments varied with the duration of haemorrhage. A vasoconstriction occurred in all vascular beds but the greatest rise in resistance was in kidneys and hind limbs. Auto regulation of blood flow in the kidneys was usually seen immediately after the first removal of blood but with the onset of renal vasoconstriction it was reduced or abolished for the remainder of the experiment.

Humoral Factors.

Humoral factors have an important role in the effectiveness of peripheral vasoconstriction. Hormonal constrictors play a dominant role in inducing vasoconstriction in skin. An important feature in irreversible shock in dogs is a progressive increase in skeletal muscle vascular conductance and this is related to an accumulative metabolic deficit, which is able to over-ride the neurogenically induced vasoconstriction (Bond, Monley and Green, 1967). Evidence in cat skeletal muscle (Mellander and Lewis, 1963) indicated that pre-capillary functions (small arteries, arterioles, and precapillary sphincters) are more under the influence of local metabolic factors than of extrinsic nervous influence. On the other hand, post capillary functions (post-capillary resistance vessels,

and main capacitance vessels) are more dominated by extrinsic nervous influence.

Buckley, Frank, Zeig, Bass and Macy (1967) demonstrated no significant difference in responses to haemorrhage between noninfused and catecholamine infused groups of dogs. Blood appeared to be shifted from the central blood volume and, although hepatic and renal blood flow decreased, the hepatic and renal fractions of the cardiac output showed no conclusively demonstrable alterations. Weidner, Albrecht and Clowes (1964) studied the effects of oligemic hypotension in unanaesthetized dogs and showed that there was evidence of anaerobic glycolysis with development of an uncompensated metabolic acidosis in all animals. This acidosis was significantly more severe among the group that died during hypotension. Manger, Nahas, Hassam, Habif and Papper (1962) showed that combination of pH control and increased oxygen delivery resulted in a greater rate of survival. It is possible that this combined treatment is associated with a better oxygen utilization.

Berne (1964) demonstrated the low oxygen content of venous blood from contracting skeletal and cardiac muscle and the close relationship between blood flow and metabolic activity in these tissues suggested a metabolically linked blood flow regulation mechanism. The chemical mediator for this type of intrinsic control of the circulation is unknown. Adenosine was suggested to be involved in regulation of coronary blood flow. Since adenosine was

not found to be present in hypoxic skeletal muscle it is unlikely to play a role in the regulation of blood flow in this tissue. Responses to respiratory acidosis were compared for the innervated and denervated kidney of anaesthetized dogs (Bersentes and Simmons, 1967). It was shown that moderate acidosis resulted in renal vasodilatation, whereas more severe acidosis caused vasoconstriction. These responses were largely independent of the renal innervation. It appeared most likely that the vasodilatation was a local effect, whereas the vasoconstriction appeared to be the result of the release of a humoral vasoconstrictor. The circulatory response is both pH and pCO_2 dependent. Goodyer (1967) suggested that irreversibility of shock is primarily determined by peripheral mechanisms, though he did not exclude cumulative myocardial damage or progressive deterioration of sympathetic drive as factors contributing to the ultimate cardiac impairment and failure observed when shock is prolonged far beyond a critical oxygen debt. However oxygen levels may not necessarily be reduced to critical levels.

Oxygen saturation in bone marrow, and in arterial venous blood during prolonged erythropoisis was studied by Grant (1948). He induced anaemia in dogs by repeated small haemorrhages and maintained this condition for 40 - 100 days. No significant difference was evident in the oxygen saturation of bone marrow in the anaemic period when compared with that of the control period.

Arterial blood showed the same constancy of oxygen saturation, but that of the jugular venous blood was decreased slightly in anaemia. The evidence indicates that a striking and prolonged stimulation of erythropoisis occurred even though the oxygen saturation of bone marrow remained at normal levels.

Rapid intravenous injection of noradrenaline caused a temporary apnoea followed by a rise in oxygen consumption (Scopes and Tizard, 1963). Intravenous infusion of noradrenaline at a slow constant rate causes a large rise in oxygen consumption, minute volume of respiration and rectal temperature in doses as small as 0·5 μg/kg/min in the unanaesthetized kittens and young rabbits. Low concentrations of oxygen in the inspired air grossly reduce the rise in oxygen consumption and rectal temperature occurring with infusion of noradrenaline. Atropine does not abolish the rise in oxygen consumption upon noradrenaline infusion. Infusion of hypertensin (angiotensin) caused a rise in blood pressure but a fall in oxygen consumption. Previous administration of hexamethonium abolished the rise in oxygen consumption on exposure to cold but not the rise in oxygen consumption and rectal temperature upon infusion of noradrenaline. Further complications are relative lung sizes in different species. Korner (1971) has shown that the rabbit is a small-lung species with a lung/body weight ratio of about 0·5% whereas man and the dog are large-lung species, with lung/body weight ratios of about 1% and a much greater capacity to increase ventilation during hypoxia.

Brooks (1935) demonstrated the reaction of chronic spinal animals to haemorrhage. The activity of the sympatho-adrenal system is attested by a post-haemorrhagic vasoconstriction, contraction of the nictitating membrane, decrease in the clotting time of the blood and a rise in blood sugar level. Removal of lateral sympathetic chains and the adrenal medulla abolished these compensatory responses. Cutting the dorsal roots of the isolated thoracolumbar cord did not abolish the ability of the spinal animal to compensate and blood pressure quickly returned to normal level after haemorrhage. Cutting the ventral roots in addition does abolish the compensatory activity. The origin of this response is in the cord. Burn and Robinson (1951) demonstrated that when a rabbit ear is perfused with Locke's solution, constriction effects are obtained by injecting small quantities of noradrenaline and of adrenaline into the perfusing fluid. The ratio of amounts of the two substances which produce the same degree of constriction is high, indicating that in these vessels the constrictor action of noradrenaline is relatively weak. After 24 hours the same procedures show similar constrictor effects for both amines. Since amine oxidase is present in the rabbit ear vessels, these observations could be explained by supposing that initially amine oxidase is very active, and its greater affinity for noradrenaline is responsible for the weak constriction action of this substance, but that after 24 hours amine oxidase activity is reduced.

Korner and White (1966) studied the circulatory control in hypoxia exerted by the sympathetic nerves and adrenal medulla in unanaesthetised normal rabbits, and in animals subjected to adrenalectomy, 'sympathectomy' (i.v. guanethidine), adrenalectomy + 'sympathectomy', and section of the carotid sinus and aortic nerves. In both arterial and primary tissue hypoxia the sympathetic nerves play a more important part in the normal circulatory response than the adrenal medullary hormones. Absence of any adrenergic activity in adrenalectomized and 'sympathectomized' animals resulted in a gradual fall in cardiac output during hypoxia, after an initial small rise. Section of the carotid sinus and aortic nerves permits maintenance of a high cardiac output during hypoxia, but the arterial pressure is low and there is probably less selective distribution of blood flow to the periphery than in animals with normal reflex control. In 1915, Morison and Hooker found the weight of an isolated loop of gut is increased in surgical shock, a fact interpreted to mean loss of local vascular tone. This loss of tone may be arterial, venous or both. Their evidence indicated loss of venous tone which would suggest failure of the veno-pressor mechanism and stagnation of venous blood. Perfusion of vascular areas, temporarily isolated for observation, showed a decreased rate of flow in shock. In traumatic shock Wiggers (1918) stated that there were two factors concerned in circulatory failure accompanying shock; a) the reduction of peripheral

resistance and b) the fall of effective venous pressure, decreasing cardiac output. The peripheral resistance, both somatic and splanchnic is at first almost invariably increased but begins to diminish when the hepatic portal pressure starts to rise (Erlanger, Gesell and Gasser, 1919).

Diana, Colantino and Haddy (1967) studied the transcapillary fluid movement during vasopressin and bradykinin infusion. Evidence is presented to show that vasopressin decreased the vascular volume increment for a given change in venous pressure whereas bradykinin increased the volume increment. An intra-arterial perfusion method was used to measure vascular resistance in hind legs of cats after acute or chronic sympathectomy (Ward, Pearson and Ederstom, 1967). Following chronic sympathectomy, vessels of intact and skinned hind limbs became supersensitive to infusion of adrenaline or noradrenaline. From the time course of the constriction and relaxation phases it did not appear that increased permeability of the blood vessels to catecholamine or its inactivation accounted for the supersensitivity.

Transfer of Interstitial Fluid.

Another compensating response involving the vascular system during haemorrhage is the transfer of tissue fluid into the circulation (Cannon, 1923). In adrenalectomized rats measurement of haemoglobin concentration in the blood during experimental hypotension (Halpern, Benacerraf and Briot 1952) indicated that in the early stage there is

a slight haemoconcentration, but in later stages there is a slight haemodilution caused probably by absorption of fluid from the extracellular compartment. In normal dogs Guyton, Batson and Smith (1951) showed that following rapid massive haemorrhage, a partial return of blood pressure toward normal occurs within the first few minutes, but total recovery is slow because interstitial fluid enters the circulation very slowly as determined by the haematocrit.

The spontaneous rate of replacement of the blood volume after haemorrhage by reabsorption of extravascular fluid was the same in normal rabbits, adrenalectomized rabbits, animals subjected to prolonged treatment with guanethidine in which peripheral adrenergic nerves transmission are blocked, animals subjected to combined adrenalectomy and guanethidine treatment with or without administration of atropine (Chalmers, Korner and White, 1967). In rabbits with carotid bodies destroyed (Grant, 1951) there was no effect on the haematocrit and reticulocyte responses following haemorrhage.

Not all workers agree that there is an increase in blood volume due to the movement of extracellular fluid. In dogs removal of 10% of initial blood volume (Gibson, Seligman, Peacock, Fine, Aub and Evans, 1947) was not followed by any significant movement of extravascular fluid, although at higher bleed volumes (20 - 50%) movement into the circulation occurred. When dogs were bled slowly until the mean blood pressure was 35 mmHg,

Huggins, Smith, Deavers and Overton (1957) showed a net increase in plasma volume and decrease in red cell volume using the ^{51}Cr. technique. Deavers, Smith and Huggins (1958) suggested that a blood pressure of about 50 mmHg appeared to be the critical pressure for operation of the compensatory mechanisms involved in the release of fluid into the circulation and for the trapping of red cells. However, early in the haemorrhagic shock in dogs the sympathetic nerves, at least with respect to skeletal muscle, act in a compensatory manner by maintaining 'venous return' through capacitance responses, and by increasing circulating blood volume as a result of the inward movement of extravascular fluid (Mellander and Lewis, 1963; Rothe and Selkurt, 1964; Haddy, Scott and Molnar, 1965). Late in the course of haemorrhagic shock, with abolition of precapillary responses, the action of the sympathetic nerves would appear to further reduce circulatory volume, in that they cause a loss of fluid from the capillaries. The haemoconcentration observed in traumatic shock differs from that of haemorrhagic shock in that the increase in filtration through the capillaries does not occur throughout the body, but is restricted to the tissue in the neighbourhood of the injury. It occurs there only during the first few hours after trauma (Engel and Forrai, 1943). In cats, reflexly induced augmentation of the vasoconstrictor fibre activity (produced by inactivation of the arterial baroreceptors, stimulation of the chemoreceptors or haemorrhage) was

found to consistently induce a net absorption of tissue fluid into the circulation from skeletal muscle and skin (Öberg, 1964). The rate of this absorption was closely related to the extent of the concomitantly occurring constriction of the resistance and capacitance vessels.

Changes in plasma protein concentration may also be involved in the tissue fluid transfer responses. The plasma electrophoretic protein pattern showed a decrease in plasma albumin concentration in both normotensive and hypertensive adrenalectomized dogs (Page and Lewis, 1951). The globulin fraction showed consistently greater shifts than other protein fractions. They were not however able to demonstrate a reduction of renin-substrate content of the blood.

Afferent Control of Sympathetic Function.

Baroreceptor and Chemoreceptors.

Alteration of both baroreceptor and chemoreceptor afferent impulses by bilateral vagotomy and carotid sinus denervation leads to the arterial pressure falling very markedly after even small haemorrhages. Since it was high before haemorrhages this indicates the importance of these reflexes in maintaining the circulation (McDowall, 1924; Remington, Hamilton, Boyd, Hamilton and Caddell, 1950; Öberg, 1964; Haddy, Scott and Molnar, 1967). Coleridge, Kenney and Neil (1949) studied the effect of blocking the vagal afferent impulses before and after selective elimination of the chemoreceptors by

injection of 0·5 N acetic acid in animals subjected to haemorrhage. The fall in arterial blood pressure caused by temporary interruption of vagal impulses was very much reduced or was no longer obtained after eliminating the arterial chemoreceptors. In 1951, Kenney and Neil demonstrated a result suggesting that the fall of arterial blood pressure caused by interruption of vagal impulses in cats and dogs during haemorrhage was essentially due to the withdrawal of aortic chemoreceptor impulses. Thus selective inactivation of aortic chemoreceptor cells by local injection of acetic acid abolished the fall of blood pressure formerly caused by vagal block. The increase in chemoreceptor discharge, just like the reduction in baroreceptor discharge, causes stimulation of the medullary cardiovascular centre and sympathetic vaso-constrictor fibres. In 1945, Bernthal, Motley, Schwind, and Week concluded that in the majority of dogs, probably in all, the thoracico-lumber autonomic outflow constitutes the sole efferent pathway for vascular reflexes originating at the carotid body. Dontas (1955) studied splanchnic outflow in the cat and in conjunction with carotid and aortic presso and chemoreceptor inflow under a variety of conditions. Prolonged hypotension due to the injection of protoveratrine coincided with a partial return of splanchnic nerve activity and tonic discharge at the presso receptors. ATP increased splanchnic nerve activity simultaneously with the hypotension and it was suggested that it should be excluded

from the list of drugs eliciting the "coronary chemoreflex."

Experiments in dogs were described by Henderson (1908) in which by the effect of a sudden great diminution in the CO_2 content of the arterial blood the heart rate was so increased that the ensuing fall in stroke output resulted in death. In 1939, Comroe suggested both carotid and aortic bodies originate reflexes involving the respiratory and vasomotor centres in response to hypoxia, whether this be produced systemically by oxygen lack in the inspired air, or locally by interference with tissue oxidation. The major role of the aortic chemoreceptors in the dogs appeared to be the initiation of powerful reflexes involving the vasomotor centre during hypoxia, the carotid body receptors being mainly concerned with respiratory reflex activation. Landgren and Neil (1951) studied the chemoreceptor impulse activity following haemorrhage. Carotid chemoreceptor discharge has been shown to be markedly increased following haemorrhage in the cat subjected to constant artificial ventilation with room air. Chemoreceptor activity thus aroused has been shown to decrease following haemorrhage, during the period of spontaneous circulatory recovery. There was also a reduction in the chemoreceptor impulses which appeared following haemorrhage during artificial ventilation with room air, when 100% oxygen was substituted for the air of the inlet side of the respiration pump. In vitro chemoreceptor discharge frequency is very low if the carotid body of the cat is bathed with saline equilibrated

with 100% oxygen. Discharge rate increases if the bathing solution is replaced by another equilibrated with 50% oxygen. Further reduction in the oxygen tension of the saline increases the discharge rate but a maximum is attained by bathing the organ with solutions equilibrated with 10 or 20% oxygen (Eyzaguirre and Lewin, 1961a).

Eyzaguirre and Lewin (1961b) further found the reduction of oxygen tension in the plasma to be the factor responsible for the activation of the carotid body chemoreceptors. Saturation of haemoglobin by oxygen appeared to play only an auxiliary role. The fall in blood pressure strongly stimulated these receptors. Raising the arterial blood pressure in cat under chloralose anaesthesia (Lee, McCloskey and Torrance, 1964), by occluding the abdominal aorta at various levels strikingly reduced the discharge of the chemoreceptors when the animal is ventilated on an O_2-N_2 mixture, but the discharge in response to hypercapnia, produced by ventilating the animal with gas mixtures containing 5 - 50% CO_2 in O_2, is little effected.

Paintal and Riley (1966) studied the responses of aortic chemoreceptors and they offered two suggestions which may explain this apparent discrepancy. First, it is possible that aortic chemoreceptors differ from those in the carotid body. The second possibility is that when combined with constant low O_2, increase in CO_2 may stimulate the peripheral chemoreceptors under conditions of greater than normal acidity but do not do this when

the blood is more alkaline. Analysis of the primary cardiovascular reflex effects of stimulation of the carotid body chemoreceptors in the dog was carried out by Daly and Scott (1962). A likely explanation of the peripheral vasodilator response occurring on withdrawing the carotid chemoreceptor 'drive' during hypoxia is therefore abolition of the primary vascular chemoreceptor reflex. This view is supported by Daly and Scott (1963, 1964) who showed that re-establishing hypoxic blood perfusion of the carotid bodies caused peripheral vasoconstriction. The primary vascular reflex from the carotid bodies may therefore be a mechanism by which the peripheral vascular resistance is maintained in systemic hypoxia, thereby representing an important function of the chemoreceptors in their control of the circulation.

Baroreceptors.

It is well known that baroreceptors discharge in response to change in tension, the impulses travelling by afferent nerves to the vasomotor and cardiac centres in the medulla (Burton, 1972). These centres on receipt of these signals send out correcting signals via the efferents of the sympathetic nervous system to the heart and blood vessels. Thus changes are produced in the controlled variable in a direction such as to reduce the error signal which started the whole process. The afferent activity has been well demonstrated. Bronk and Stella (1932) reported a discharge of afferent impulses

from pressure receptors in the carotid sinus of the rabbit at all pressures found in normal living animals. The degree of activity varied with the blood pressure and is to a considerable extent dependent upon the variations in arterial pressure during the heart cycle.

Decrease of both pulse pressure and mean arterial pressure reduce the afferent impulses from the baro-receptors in the carotid sinus and aortic arch (Eade, Green and Neil, 1952). Beck and Dontas (1955) reported that during the initial bleeding period, as the blood pressure was reduced to the desired hypotensive level, baroreceptor activity was either markedly reduced or disappeared altogether in both dogs and cats. Surprisingly in their dogs activity usually increased slightly as secondary bleeding took place and decreased as automatic re-infusion from a blood pressure compensator occurred. Chemo-receptors in cats do not appear responsive unless the mean blood pressure has been reduced to 45 - 60 mmHg. In dogs the chemoreceptors seldom became very active until just before death. However Coleridge and Kidd (1963) reported that in eight out of eighteen dogs pulsatile distension of the right pulmonary artery which was converted into a closed sac (mean pressure between 20 and 60 mmHg) produced systemic hypotension and some-times bradycardia. These effects were augmented by carotid occlusion and abolished by cooling (7 - 8^{o}C) or section of the vagus nerves. It was concluded that the hypotension was reflexly brought about by activation

of pulmonary arterial baroreceptors. In cats, electrical stimulation of the afferent fibres in cardiac sympathetic nerves always elicited a pressure response during the stimulation of the central end of the cut left inferior cardiac or pericoronary nerve in vagotomized, intact-brain or spinal cats (Peterson and Brown, 1971). The alpha-blocking agent phenoxybenzamine abolished this pressor response. This result was in agreement with the findings of Brown and Malliani (1971), that changes in coronary flow in spinal, vagotomized cats evoked a reflex increase in sympathetic efferent discharge.

James (1971) studied the effects of altering mean pressure, pulse pressure and pulse frequency on the impulse activity in baroreceptor fibres from the aortic arch and right subclavian artery in rabbits. At low initial mean pressure, an increment of pressure, at constant pulse pressure and frequency, increased the total impulse activity by increasing the frequency of impulses in single fibres already active during systole and diastole and by additional recruitment of other fibres. At high mean pressures there is little increase in impulse activity as the maximum frequency of fibres is attained or superseded and there was little recruitment. James and Daly (1971) isolated the carotid sinuses and aortic arch in anaesthetized dog and separately perfused it with blood by a method which enabled the mean pressure, pulse pressure and pulse frequency to be varied independently in each vasosensory area. They confirmed that under steady-

state conditions the vasomotor responses elicited reflexly by changes in mean carotid sinus pressure are modified by alterations in the carotid sinus pulse pressure. Whereas those evoked by changes of mean aortic arch pressure are only weakly affected by modifications of aortic pressure.

The Vasomotor Centre in Haemorrhage.

The medullary cardiovascular centre itself might be susceptible to tissue ischemia during haemorrhage.

Seelig and Lyon (1910) assumed that augmentation of the cardiac activity in shock is brought about through impulses delivered along the accelerator nerves. These accelerator fibres, passing as they do through the stellate ganglia, could be inactivated by removal of these ganglia. Their results showed in every instance, that changes in shock were not due to a vasomotor exhaustion. In 1914, Pilcher and Sollmann reported that haemorrhage progressively stimulates then depresses and paralyses the vasomotor centre. The period of stimulation was variable, but usually persisted during a total haemorrhage of about 25 cc per kg when the blood pressure has fallen to about 90 - 100 mmHg. A period of vasodilation followed the stimulation. The perfusion flow either remained below normal, returned to normal, or increased somewhat above the normal. The centre became paralyzed when about 35 - 40 cc per kg had been withdrawn and when blood pressure had reached a very low level (approximately 30 mmHg).

Catecholamine Release from the Adrenal Medulla during Haemorrhage.

Haddy, Scott and Molnar (1965) studied the effects of haemorrhage in dogs and reported a baroreceptor induced sympathico-adrenal discharge and parasympathetic inhibition which a) opposed the fall in cardiac output by increasing the rate and strength of cardiac contraction and by aiding venous return (redistribution of remaining vascular volume subsequent to splenic contraction and possibly hepatic and venous contraction), and b) raised peripheral resistance by actively constricting arterioles and transiently increasing blood viscosity due to splenic discharge.

In all animals the medullary tissue is of neuroectodermal origin arising from the same embryonic tissue, the neural crest, that generates the sympathetic ganglia (Orth, 1973). The secretory cells are, in fact, postganglionic elements of the sympathetic system and remain synaptically associated with the preganglionic fibres. Burn (1956) discussed the physiology of the adrenal gland but could not explain why the cortex surrounds the medulla in the adult mammal. He showed the difference in action between adrenaline and noradrenaline on the vascular system. Adrenaline causes vasoconstriction in the skin and intestinal region, but in moderate amounts it caused dilatation of the skeletal muscle vessels. This dilatation is of obvious value during an emergency situation when a large increase in blood supply to the muscles is required.

Noradrenaline caused constriction in all vascular beds including muscle vessels. Shepherd and West (1951) suggested that in the embryonic adrenal glands of cat, rabbit, Guinea-pig, dog and man the main hormone released is noradrenaline, very small amounts of adrenaline being found. In the adult glands of these mammals, it is suggested that the degree of methylation of noradrenaline is related to the relative sizes of the cortex and medulla. In animals where the cortex is large relative to the medulla (e.g. rabbit and Guinea-pig) methylation of noradrenaline is almost complete, and often only adrenaline is found in gland extracts and perfusates. In the rabbit the adrenal gland secretes only adrenaline during splanchnic nerve stimulation (Powis, 1974). The probable effects of endogenous adrenaline released at each frequency of splanchnic nerve stimulation on the vasculature of the hind limb of the rabbit can be derived from the results of adrenaline infusion. Adrenaline exerts only a constrictor action on the hind limb vasculature. Noradrenaline produced smaller vascular changes than did adrenaline at any individual dose level.

The activity of the adrenal medulla and its regulation was the subject of an extensive review by Malmejac (1964) who concluded that the preganglionic innervation of the adrenal medulla is cholinergic, the neuro-adreno-medullary junction behaving like any sympathetic ganglionic synapse in terms of transmitter excitation, chemical interruption of transmissions and measurement of facilitating transmission.

Generally the adrenal medulla releases both pressor catecholamines, adrenaline and noradrenaline, into the circulation. The proportion of the latter varies according to the animal species.

The release of catecholamines from the adrenal medulla by its normal transmitter, acetylcholine, has been studied by Douglas and Rubin (1961a, 1961b). They suggested that acetylcholine caused a brief change in the medullary cells which allows calcium ions to penetrate them and trigger the catecholamine ejection process. These results have been further supported by Douglas and Rubin (1963).

In dogs under physiological conditions of activity, hypotension, hypoglycaemia or hypoxia the amounts of adrenaline or noradrenaline released are not specifically related to the particular condition. It seems unlikely that in this animal two distinct nervous pathways separately regulate the activity of adrenomedullary cells some containing adrenaline and others noradrenaline. Malmejac suggests that circulating noradrenaline is largely of sympathetic adrenergic nerve origin while adrenaline is almost all of adrenomedullary origin.

Darrow and Gelhorn (1939) reported that the injection of adrenaline or the liberation of adrenaline from the adrenal medulla leads to diminished reflex excitability of the sympathetic nervous system. The blood pressure response to stimuli applied to afferent nerves is diminished or disappeared completely, the contraction of the

innervated nictitating membrane is lessened and the difference between reflex pupillary dilatation on the normal and sympathectomized sides following afferent stimuli is diminished or reversed. The experiments thus indicate an inhibitory action of adrenaline on mechanisms controlled by both branches of the autonomic nervous system. By perfusing a cat's adrenal gland (Douglas and Poisner, 1966) with Locke's solution and stimulating the splanchnic nerves or by perfusing acetylcholine, in addition to the catecholamines, large amounts of AMP and adenosine as well as smaller amounts of ATP and ADP appeared in the venous effluent. That these substances had their origin in the chromaffin cells was suggested by their failure to appear when the splanchnic nerves were stimulated following perfusion with drugs that block the adrenal synapse (hexamethonium and atropine). It was concluded that the nucleotide rich granules are the immediate source of catecholamines released from the stimulated adrenal chromaffin cells. Critchley, Ungar and Welburn (1973) studied the selective release of catecholamine in anaesthetized cats and dogs. They found that reflex responses were elicited by lowering the carotid perfusion pressure (baroreceptor test) and by lowering the pO_2 of blood perfusing the carotid region without affecting that of systemic arterial blood (chemoreceptor test). Their results confirmed that the reflex response to chemoreceptor stimulation in the cat is predominantly a release of noradrenaline. In the dog,

there was no selective response. Both tests gave rise to release of adrenaline and noradrenaline in about the same ratio as in resting secretion.

In 1977, Helle and Serck-Hanssen showed that the bovine adrenal glands *in vitro* also released a higher proportion of noradrenaline in response to stimulus by acetylcholine than in the control periods. The release of adrenaline was maintained at the same level during the successive periods of stimulation while the release of noradrenaline declined in parallel with the decline in dopamine β- hydroxylase activity. These results suggested that noradrenaline and adrenaline granules contribute differently to the release of active dopamine β- hydroxylase during repetitive stimulations.

It is well known that haemorrhage causes reduction in total blood volume, this being detected principally by atrial type B receptors (Milnor, 1974). A reflex sympatho-adrenergic discharge increases catecholamine secretion. In anaesthetized dogs Bedford (1917) inserted a cannula into the inferior mesenteric vein, and pushed it in until its tip was well into the vena cava, opposite the opening of the right adrenal vein. By using a rabbit intestinal strip *in vitro* for estimation of adrenaline he concluded that adrenaline increased in the blood during haemorrhage and this increased amount of adrenaline is accompanied by a hyperactivity of the adrenal gland and is not simply the result of the release of adrenaline material stored in the gland. The

adrenaline content of the blood increased only after prolonged reduction of the blood pressure. Other workers have shown a much earlier release of catecholamine in haemorrhage. Watts (1956) used arterial blood of dogs to show quite clearly that as haemorrhage progresses and blood pressure falls, the adrenaline content of the blood, indicated by *in vitro* uterine strip inhibition, increased. There were definite indications that the adrenaline content of the blood reached a maximum soon after blood pressure was lowered to a level at which shock occurred and was disappearing from the blood at the time of death from shock.

In 1957, Watts and Bragg found the adrenaline content of both arterial and venous blood increased during haemorrhage, the concentration in venous blood increased to a maximum of 12·5 μg/l (control value; 1 μg/l). The adrenaline level decreased during automatic reinfusion while maintaining the blood pressure at 40 mmHg, and reached a level of 5·2 μg/l during the later period of reinfusion before the dog died. Millar and Benfey (1958) using a fluorimetric method for estimating adrenaline and noradrenaline during haemorrhagic hypotension in anaesthetized dogs, demonstrated a marked increase in plasma and urinary adrenaline concentration in response to progressive reduction of blood pressure. They concluded that one early component of the sympatho-adrenal response to haemorrhagic shock is the secretion of adrenaline, presumably from the adrenal medulla; this marked by

dominating a more later rise in the plasma level of noradrenaline, secreted from the adrenal medulla or from sympathetic nerve endings. The increase in concentration of catecholamine (adrenaline rather than noradrenaline) in the adrenal vein blood was found to be in proportion to the fraction of the total blood volume removed (Walker, Sherefettin Zileli, Reutter, Shoemaker, Friend and Moore, 1959). The peripheral arterial blood concentration of adrenaline was higher than the venous blood and appeared to be more closely correlated with the concentration in adrenal venous blood. These findings were in agreement with those of Poole and Watts (1959) who showed that adrenaline is rapidly inactivated when it is brought into intimate contact with tissue. The difference in values from blood collected from the femoral artery and vein represented inactivation of adrenaline in the hind leg of the dog which is mainly muscle. Observations made by Watts and Westfall (1964) showed that during haemorrhagic shock in dogs the circulating catecholamines increased as the pressure was lowered below 80 mmHg. This increase is largely due to adrenaline and to a lesser extent to noradrenaline. Simultaneous estimation showed adrenaline concentration to be greatest in the inferior vena cava followed by the femoral artery and lowest in the femoral vein. These results indicated that most of the catecholamines in the circulation during haemorrhage in the dog are released from the adrenal medulla and are rapidly destroyed by the tissue.

Greever and Watts (1959) showed that arterial adrenaline levels in dogs increased from control values during haemorrhage and decreased during the period of spontaneous reinfusion. After completing the reinfusion the blood pressure returned to normal, and adrenaline disappeared from the circulation. Ganglionic blockade with hexamethonium led to an unchanged blood level of adrenaline and noradrenaline during the early part of haemorrhagic hypotension in dogs (Walton, Richardson, Walton and Thompson, 1959). With hexamethonium and mecamylamine, the same general effect was observed but the sympathetic responses were less severely reduced. Adrenaline levels at relatively late stages of the experiments were significantly greater in the series in which haemorrhage was unmodified by ganglionic blockade as compared to the series using hexamethonium.
Glavians, Bass and Nykiel (1960) diverted blood from the left adrenal vein to a cannulated left external jugular vein of the same animal. A differential biological assay method using a rectal caecum of a hen for the adrenaline determination and the pressor effect on the blood pressure of anaesthetized cats for noradrenaline determination was used during haemorrhagic hypotension in the dog. The data obtained on adrenal catecholamines secreted in haemorrhagic hypotension has several interpretations. One of their observations was concerned with the adrenals remaining functional to the point where the animal is within minutes away from complete cardio-respiratory collapse.

They concluded from these and other experiments that the high levels of adrenaline in peripheral blood do not result, at least to any large degree, from a depression of physiological processes for deactivation of the catecholamine. On the contrary, the adrenals consistently demonstrated an amazing capacity to secrete continuously pressor hormones under the severe stress of progressive circulatory failure. Hypotension of varied level and duration was observed to be primarily accompanied by a highly significant increase in adrenal vein plasma adrenaline. On the other hand, the secretion of noradrenaline had an unpredictable occurrance in adrenal blood samples collected during periods of hypotension. When anaesthetized dogs were bled to a blood pressure of 80 - 90 mmHg the left adrenal catecholamine output was augmented from 2·9 to 7·4 times the control levels (Fowler, Shabetai and Holmes, 1961). When dogs were bled to 40 - 50 mmHg the output was augmented between 1·8 and 17·0 times control values.

Following the onset of haemorrhage in anaesthetized dogs, even more striking increases in the peripheral venous levels of endogenous adrenaline were noted by other workers. Rosenberg, Lillehei, Longerbeam and Zimmermann (1961) showed a spectacular 90-fold increase over control values. Thereafter levels decreased but never approached normal resting concentrations. Following re-infusion of shed blood, the catecholamines decreased in the same striking fashion that they increased.

A report made by Manger, Nahas, Hassam, Habif and Papper (1962) has shown that the increase in plasma catecholamine concentration in dogs receiving saline transfusion after haemorrhage was twice as large as in those receiving a buffer solution. In all animals the increase in adrenaline was considerably greater than noradrenaline. During haemorrhagic shock, they suggested that if catecholamines are to exert their optimal activity during haemorrhagic shock, adequate oxygen supply and normal blood pH are essential requirements. In 1964, Darby and Watts again showed an increase in blood levels of adrenaline and noradrenaline during periods of haemorrhagic hypotension in dogs. They noted an increasing metabolic acidosis which paralleled the rise of plasma catecholamines. They further showed that blockade of the sympathetic nerve supply significantly reduced the circulating levels of catecholamines during the early stages of haemorrhage and this was accompanied by a lesser degree of acidosis.

The relation of myocardial function to survival after oligemic hypotension has been studied on unanaesthetized dogs in a normal basal state (Weinder, Albrecht and Clowes, 1964). During the period of hypotension, some of the animals which died, either before or after the reinfusion, exhibited a significantly higher blood catecholamine concentration than the survivors. During hypotension there was evidence of anaerobic glycolysis with development of an uncompensated metabolic acidosis

in all animals. This acidosis was significantly more severe among the groups that died.

Regoli and Vane (1966) superfused a rat stomach strip and chick rectum continuously by blood drawn from the carotid artery of anaesthetized dogs and observed after a small haemorrhage there was no significant release of catecholamine. Longer haemorrhages induce the secretion of catecholamine which could be inhibited by ganglionic blockade. In cat and dog at slow rates of haemorrhage, catecholamines rose in the cat but not in the dog; at fast rates, both species showed a rise, but this was higher in the cat (Hall and Hodge, 1971). During slow progressive haemorrhage in dogs, the appearance of angiotensin preceded that of catecholamine. Anaesthesia affects the catecholamine output (Walker, Sherefettin Zileli, Reutter, Shoemaker and Moore, 1959; Hume, 1961), the release during deep levels of anaesthesia being lower than that during light anaesthesia.

Celander (1954) has suggested that the primary action of the medullary secretion is the sympathetic hormonal control of various metabolic processes in tissues which lack a direct sympathetic innervation. He suggested a clear-cut functional differentiation between the neural and the humoral fraction of the 'sympathico-adrenal system.'

In the dog, with all the intestine except the distal half of the colon denervated, the only part which showed haemorrhagic necrosis was the innervated portion

(Fine, 1965). This he suggested was proof that noradrenaline released in the tissues, rather than the circulating catecholamine was responsible for tissue injury, and therefore, for the progressive vascular collapse. These results confirmed previous observations of Zetterstrom, Palmerio and Fine (1964) who denervated half the spleen of the dog three weeks before carrying out bleeding procedures. They observed the spleen until the death of the animal. The non-denervated half contracted as soon as bleeding began and remained contracted until the dog was re-transfused 6 hours later, at which time it returned to normal size. The denervated half of the spleen did not change in size, shape, or colour, at any time and responded to intravenously administered noradrenaline until death.

In rabbits, West (1951) found that when insulin was injected in sufficient amounts the activity in the suprarenal medulla first increased and then decreased. Noradrenaline in small amounts may appear in extracts of suprarenal gland only during the first two hours after the injection. At other times the glands contained only adrenaline. Holzbauer and Vogt (1954) demonstrated a rise in the adrenaline concentration of the plasma which commenced a few minutes after the intravenous injection of insulin and lasted for several hours both in dogs and in human subjects. The adrenaline concentration increased when the dose of insulin was raised. No noradrenaline was found in any of the plasma samples

either before or after the injection of insulin. Fowler, Shabetai and Holmes (1961) reported increased adrenal medullary secretion during hypoxia in anaesthetized dogs. This increase was not dependent upon a fall in systemic arterial blood pressure and was not associated with an increase of 17-hydroxycorticosteriod output.

Inducing hypoxia in the concious calf for 8 - 10 minutes no significant change in plasma insulin concentration was observed (Bloom, Edwards, Hardy, Malinowska and Silver, 1976; Broom, Edwards, Hardy and Silver, 1976). Thus it is unlikely that insulin mediated release of catecholamine from the adrenal medulla is an important factor during hypoxia. The release of adrenal steroids was reflected in the concentration of cortisol and corticosterone in the arterial plasma accompanied by a pronounced increase in blood flow through the gland. Secretion of catecholamines from the adrenal medulla was not observed until the pO_2 of the arterial blood had fallen below 15 mmHg. However, hypoxia of this severity stimulated release of both adrenaline and noradrenaline. They concluded that, although the adrenal medulla remains insensitive to a relatively large reduction in the arterial pO_2, intense hypoxia represents a maximal stimulus for the release of both glucocorticoids and catecholamines from the adrenal medulla.

The Renin - Angiotensin System in Haemorrhage.

During haemorrhage there is increase in release of renin as a direct response to a reflex increase in sympathetic activity induced by a reduction of blood pressure or volume. Renin is not itself a pressor substance; it is a proteolytic enzyme released from the juxtaglomerular apparatus of the kidney, which hydrolyses angiotensinogen, an α_2-globulin fraction of the plasma protein produced in the liver, to give a vasoconstrictor substance called angiotensin. Angiotensin, in small amounts increases the rate of aldosterone production from the adrenal cortex which stimulates reabsorption of sodium from the tubular fluid of the nephron and acts to compensate further for the reduction in blood pressure or volume.

Renin has been assayed in the plasma of unanaesthetized humans, dogs (Brown, Davies, Lever, Robertson and Tree, 1964) and rabbits plasma (Lever and Robertson, 1964; Ryan, McKenzie and Lee, 1968) under normal resting conditions.

The kidney is the main source of the enzyme renin in rabbits and other species of animals, and a stable preparation of renin has been made by drying and powdering the residue from rabbits kidneys treated with alcohol (Pickering and Prinzmetal, 1938). Some workers have suggested other sources for renin and a pressor material has been isolated from the rabbit's uterus with several characteristics of renin (Ferris, Gordon and Mulrow, 1967a, 1967b). The concentration of renin in

the pregnant uterus was greater than in the non-pregnant uterus and was equal to that of whole kidney but because of the greater weight of the pregnant uterus it was clearly a greater potential source of renin than the kidney. The same findings were reached by Ryan and Ferris (1967) who demonstrated that the incubation of the renin-like enzyme of pregnant uterus (in vitro) with renin-substrate gave a vaso-pressor product which was probably angiotensin I. This led to the suggestion that it could form angiotensin II in vivo and may effect sodium homeostasis and cardiovascular function in the pregnant female. However, the finding of Lumbers (1973) strongly suggests the kidneys in the female rabbit to be the major source of plasma renin and hence angiotensin, although extrarenal renin from the uterus may exist.

In 1938 Kohlstaedt, Helmer and Page studied the vaso-constrictor action of renin prepared from pig's kidneys on perfused dogs'tails, so eliminating all factors causing vasoconstriction other than peripheral ones and from the results they got, suggested that renin is an enzyme-like substance which is activated by a kinase-like material contained in the protein fraction of plasma and whole blood. The fluorescent antibody technique in the rabbit, dog and domestic pig, demonstrated that renin is located in the juxtaglomerular cells, but not in the macula densa or other structures of the renal cortex (Hartroft, 1963). In normal isolated dog's kidney perfused with

blood the renin appears to be produced when pulse pressure and blood flow are reduced by constricting the renal artery (Kohlstaedt and Page, 1940a). Reduction of mean pressure is not a necessary condition. Renin is not produced, for example, when the hind leg is perfused under similar circumstances in which the arterial inflow is constricted, nor by kidneys perfused with blood under normal pressure flow conditions. It is possible to demonstrate increased liberation of renin from the kidneys into the renal venous blood by addition of renin activator and perfusion of the mixture through isolated organs (Kohlstaedt and Page, 1940b). Dog's kidney perfused under what appeared to be normal haemodynamic conditions liberates little renin. Reduction of pulse pressure appeared to be the main stimulus for renin release. Reduction of blood flow followed but appeared to be an effect rather than the cause of the increased liberation of renin.

The hypertension induced by narrowing the calibre of the renal artery in dogs by means of a clip (Goldblatt, Lynch, Hanzal and Summerville, 1934), caused an ischemia localised to the kidneys and is a sufficient condition for the production of persistently elevated systolic blood pressure. When only moderate constriction of both main renal arteries is carried out, the elevation of systolic pressure is unaccompanied by signs of materially decreased renal function. Page (1935) concluded from experiments on dogs that the renal nerves do not appear to participate

in the genesis of hypertension and hypertension produced by constriction of the renal arteries does not result in significant changes in the proteins or lipids of the plasma. The haemoglobin content of the blood is slightly elevated, renal efficiency is not markedly altered and bears no relation to the increase of the blood pressure. However, oxygen consumption by slices of rabbit renal tissue studied by means of the Warburg manometric method (Gerbi, Rubenstein and Goldblatt, 1940), indicated that the ischemic kidney showed a definite reduction of oxygen consumption compared with the normal kidney. A decrease in activity of various tissue enzymes in the hypertensive rats, especially a disturbance of the cellular oxidation system in the hypodynamic kidney and the over worked heart in hypertension have been demonstrated by Ruskin, Hall, Ruskin and Hall (1953). These results were in contrast to those of Hass and Goldblatt (1959) who showed a considerable increase in the concentration of renin, itself an enzyme, in the ischemic kidney.

The results obtained from the experiments of Walkerlin and Chobot (1939) on dogs suggested no evidence at that time for a possible role of renin in maintenance of normal blood pressure. These workers concluded that bilateral nephrectomy increased the sensitivity of the dog to the pressor action of renin administered intravenously. The renin content of the kidney bore no relation to the blood pressure level of the animal, neither did stimulation of splanchnic nerve nor hypotension alter significantly

the renin concentration of the kidney. House and Wakerlin (1941a, 1941b) have shown in their experiments no significant difference between the decrease in blood pressure of nephrectomized and sham nephrectomized dogs following splanchnectomy or chordotomy. They suggested that the kidney does not play a specific role in the regulation of normal blood pressure in the dog and suggest that renin, like adrenaline and ADH, does not exert a pressor effect under normal conditions. However, observations made on dogs with renal artery constricted (Cowley, Miller and Guyton, 1971) have shown that systemic pressure changes were inhibited by infusion of angiotensin II before constriction and by the injection of angiotensin II antiserum. The results, the authors suggest, indicate that the renin-angiotensin system possesses sufficient time response and gain characteristics to participate significantly in the normal regulation of arterial pressure. Renin was detected in the plasma of all normal rabbits studied by Lever and Robertson (1964). When the rabbits were made hypertensive by the application of a renal artery clip, significantly higher renin levels were found than in a group of normal rabbits, while renin was greatly reduced in the plasma of rabbits from which both kidneys had been removed 24 hours previously. When mean renal perfusion pressure was reduced from 5 to 40 mmHg by a constricting band around the aorta above the level of the renal arteries of anaesthetized dogs (Skinner, McCubbin and

Page, 1964) the renin secretion increased within 60 seconds. Reduction of pulse pressure alone did not provoke secretion of renin, nor did reduced oxygen tension, nor renal ischemia. Renin secretion was found to be controlled by a renal baroreceptor rather than by ischemia (Skinner, McCubbin and Page, 1963). Coote, Johns, MacLeod and Singer (1972) studied the effect of renal nerve stimulation, renal blood flow and adrenergic blockade on plasma renin activity in the cat. They indicated that renal blood flow changes are of prime importance in mediating the action of renal nerves on renin release, and that a propranolol-sensitive step is involved. It is clear that control of renin release is considerably more complicated than originally visualized (Vander, 1967). On the ~~other~~ one hand there exists a wholly intrarenal mechanism, be it baroreceptor, macula densa or both, capable of altering renin release. On the other hand there also exists a more complex reflex control pathway that may predominate under usual physiological conditions. The findings of Sapirstein, Ogden and Southard (1941) from experiments on dogs appeared to show the renin content of blood increases after haemorrhage since it caused contraction of the Guinea-pig ileum used for the estimation. Prehaemorrhagic blood, which according to this concept, contained no renin, was incapable of sensitizing the gut. These findings support the view that the kidney acts as an organ of internal secretion, preserving the homeostasis of the whole circulatory

system by a humoral mechanism. This thesis was supported by Hamilton and Collins (1941); Huidobro and Brawn-Menedez (1942) who showed that the kidney participates in the regulation of arterial blood pressure. When the blood pressure decreased the normal kidney secretes renin which through the formation of angiotensin II acts to restore normal blood pressure. They suggested that renin is probably of major importance in the normal maintenance of cardiovascular homeostasis.

Hamilton and Collins (1942) support the previous results, their findings indicate that both haemorrhage and histamine evoke the release of a humoral substance from the kidney and that this mechanism aids in the maintenance of arterial blood pressure. Their findings also suggested that the kidneys could liberate enough renin to cause tachyphylaxis and thus reduce their own compensatory effects.

Dexter, Frank, Haynes and Altschule (1943) studied the plasma concentrations of renin and angiotensinogen in unanaesthetized dogs during haemorrhagic shock. They confirmed that readily detectable amounts of renin appear in the circulating plasma. The concentration of angiotensinogen decreased in severe haemorrhagic shock, sometimes to extremely low levels. The renal humoral pressor mechanism was considered to represent a compensatory reaction on the part of the body, to maintain normal blood pressure following haemorrhage. Hamilton and Collins (1944) demonstrated an early increase of

renin-substrate during haemorrhage in both intact and adrenalectomized dogs, which was followed by a significant reduction following varying periods of hypotension. At the same time nephrectomized dogs subjected to hypotension failed to show significant rises in angiotensin-like substances. Bled intact dogs consistently showed increase in a substance which contracted the ileum of the Guinea-pig, elevated blood pressure in dogs and cats, and caused vasoconstriction in the rabbit's ear. This substance is presumed to be angiotensin. A secondary fall in endogenous angiotensin occurred subsequent to the depletion of renin-substrate. Scornic and Paladini (1964) demonstrated increase in angiotensin blood levels of dogs in haemorrhage and constriction of the aorta above the level of renal arteries. They suggested but did not prove that this rise in angiotensin blood level after haemorrhage may be due to an extreme reduction in renal blood flow, which promotes the liberation of renin and/or an increase of its action. Regoli and Vane (1964b) demonstrated contraction of the rat colon within 2 minutes after reducing the blood pressure by haemorrhage. They suggested that the contraction was due to an increase in circulating angiotensin, generated by the release of renin from the kidneys, for the following reasons:-

(1) The contraction disappeared after nephrectomy.

(2) The concentration of the active substance was higher in renal venous blood than in carotid arterial blood. (3) The responses of the rat stomach strip

and chick rectum which were used together with the rat colon were similar to those produced by ∝ - hypertensin (angiotensin II). (4) The decay of the contraction after reinfusing the blood was slower than the decay after an i.v. infusion of ∝ - hypertensin, but similar to that after an i.v. injection of angiotensin. Haddy, Scott and Molnar (1965) suggested renal ischemia might slightly raise peripheral resistance through production of angiotensin. Late responses which may compensate for the fall in blood pressure are: a) increased renal tubular reabsorption of fluid via the ADH and renin-angiotensin-aldosterone systems which oppose the fall in cardiac output by minimizing loss of blood volume, and b) reduction in blood viscosity which reduces the fall in cardiac output by lowering the resistance to filling of the ventricles.

Brown, Davies, Lever, Robertson and Verniory (1966) showed plasma renin concentration in the dog and man were increased after the larger bleeds; after smaller haemorrhages plasma-renin concentration remained unchanged. Hodge, Lowe and Vane (1966) studied the effects of alterations of blood volume on the concentration of circulating angiotensin in anaesthetized dogs. They showed that haemorrhage caused inverse changes of angiotensin concentration with blood volume owing to changes in the rate of generation of angiotensin. This was probably due to changes in the rate of secretion. The renal arterial or venous pressure was not responsible

for the changes of angiotensin generation rate. They were abolished by blocking the renal nerves with lignocaine. There was a consistent inverse correlation between angiotensin levels and central venous pressure but not with systemic arterial pressure. It was concluded that changes of blood volume bring about changes of the rate of generation of angiotensin by a reflex mechanism the efferent limb of which involved the renal nerves. The afferent pathway remained unknown, but the systemic baroreceptors did not appear to be of primary importance. The renin-angiotensin system is important in the homeostatic response to changes of blood volume. In 1966, Regoli and Vane demonstrated the appearance of angiotensin in the circulation of the dog after a small haemorrhage without a concomitant release of catecholamine while with a longer haemorrhage, catecholamine as well as renin was demonstrated in the circulating blood. The catecholamine secretion was inhibited by ganglion block, but renin secretion was not. It was concluded that the secretion of renin by the kidneys in response to a fall of renal blood pressure is a physiological response, probably of importance in homeostasis.

Hall and Hodge (1971) demonstrated a consistent rise in angiotensin levels in both dogs and cats during haemorrhage. During slow, progressive haemorrhage in dogs, the appearance of angiotensin preceded that of catecholamines. It is concluded that the renin-angiotensin system is important in circulatory haemorrhage

in both species.

Davis and Freeman (1976) studied the mechanisms regulating renin release and reported that the initial stimulus to renin release is a general decrease in blood volume. In small non hypotensive haemorrhage, the increase in renin secretion is mediated by the renal sympathetic nerves. In addition, such haemorrhage increased catecholamine release from the adrenal medulla. A large hypotensive haemorrhage might activate both extra-renal and intra-renal pathways, the latter assuming more importance. If the effects of haemorrhage are analysed in terms of an automatic control system, haemorrhage does not open the control loop. Instead, the increase in sodium reabsorption expands the blood volume and restores arterial pressure and renal blood flow as compensation occurs.

In anaesthetized rabbits, the arterial plasma renin activity rose rapidly to between two to three times the control value by the end of severe 2 - 3 minutes haemorrhage (McKenzie, Lee and Cook, 1966). Their rabbits were bled to a low pressure which varied from 25 - 40 mmHg.

The Interaction of Angiotensin and other substances with the Adrenal Medulla.

The probable interaction between the renin-angiotensin and adrenal medullary hormones has been suspected for many years.

Probably the work of Feldberg (1940) was an important landmark. He showed that in cats, bee venom and cobra venom caused a large lasting output of adrenaline from the adrenals if injected into the central stump of the coeliac artery. The effect has been attributed to the formation of lysolecithin in the tissue, since lysolecithin was found to cause an output of adrenaline similar to that produced by the venoms. In rabbits lysolecithin has either no effect or only a slight inconstant secretory action on the adrenal medulla. Infusions of DMPP (1,1-dimethyl-4-phenyl-piperazinium iodide), a ganglionic stimulating agent caused increase of pressor responsiveness to angiotensin. Instead of enhancement of the direct effect of angiotensin on the cardiovascular system, the larger responses that follow treatment with DMPP are due to adrenal discharge of what were presumed to be catecholamines (Kaneko, McCubbin and Page, 1961). Other ganglion stimulating agents "sensitize" the adrenal in the same manner, and a variety of vasoconstrictor drugs elicit discharge of hormones from the sensitized gland. They concluded tentatively that ganglion stimulating agents cause sensitization of receptors in or near the adrenal glands which, through

a local neural mechanism containing a cholinergic synapse and an adrenergic receptor, respond to hypoxia by causing discharge of catecholamines. According to these authors, however, the release was not due to a stimulating action of angiotensin on the medullary cells, but was a consequence of its vasoconstriction action. Feldberg and Lewis (1964) showed that bradykinin and angiotensin are potent releasers of the medullary hormones, probably mainly adrenaline, from the suprarenal glands. Angiotensin was more potent than any other known substance. The experiments were performed on anaesthetized, eviscerated cats, the injection of the peptide being made into the central stump of the coeliac artery. They suggested that the release of the medullary hormone which caused contraction of the cat's denervated nictitating membrane was due to stimulating action of angiotensin either directly on the medullary cells or through excitation of the cholinergic pre-ganglionic sympathetic nerve endings in the medulla. They also queried the possibility of a direct pathway from the kidney to the suprarenal gland, perhaps via the lymphatic vessels, so that angiotensin could act on both cortex and medulla without having first to enter the systemic circulation. Angiotensin some times released as much as a hundred times its own weight, of catecholamines when injected through the coeliac artery while when injected intravenously into a non-eviscerated anaesthetized cat the release of the medullary hormones was obtained with as little as 0·1μg of angiotensin.

Further study of the release of catecholamines from the adrenal medulla by peptides was carried out in cats and dogs by Staszewska-Barczak and Vane (1965). They demonstrated that intravenously injected peptides were about one-tenth as potent as those injected intra-arterially. Angiotensin was the most potent peptide in the cat but it was less potent than kallidin and bradykinin in the dog. In neither the cat nor the dog was the effectiveness of the peptides decreased by ganglion-blocking agents. Moreover, in both cat and dog, angiotensin (2 - 50 μg/min) caused an initial burst of adrenaline secretion. This rapidly declined during the infusion and within 5 - 10 minutes the secretion stopped; additional injections of angiotensin producing no further response. They suggested that, because there is rapid adaptation of the adrenal medulla to constant concentrations of angiotensin, its effect on the medulla is unlikely to be important in homeostasis. Furthermore, since there was no cross-tachyphylaxis, they infer that the receptors in the adrenal medulla for angiotensin are different from those for bradykinin and kallidin.

In 1965 Feldberg and Lewis made a further study on the effects of peptides on the suprarenal medulla of cats. The release of catecholamines was detected by their action on the denervated nictitating membrane. They demonstrated that the peptides do not act on the cholinergic nerve endings of the adrenal glands, when releasing the hormones, but on receptors in the medullary

cells different from those activated by acetylcholine. Renin which forms angiotensin, and several analogues of angiotensin, was also found to release the medullary hormones. They also showed that hexamethonium which renders the cells insensitive to stimulation by acetylcholine does not reduce their sensitivity to the injected peptides. After removal of the suprarenal glands relatively large doses of angiotensin given intravenously produced a delayed contraction of the denervated nictitating membrane. Their contraction was not due to a direct action of angiotensin on the membrane since it did not occur on injection into the carotid artery. They suggested it results from an action of catecholamine released either from the adrenergic nerve endings or from extra-medullary chromaffin tissue. Many other results add weight to the evidence for the release of adrenal catecholamines by vasoactive peptides.

After blocking the β -adrenergic bronchodilator receptors with pronethalol and propranolol (Nagasaka, DeSchaepdryver and Heymans, 1964a, 1964b) the intensity of bronchoconstriction induced by a maximally effective dose of bradykinin was increased and the dose needed for a given submaximal effect was reduced. Bilateral adrenalectomy had a similar effect. Collier, James and Piper (1965) supported the conclusion that bradykinin and angiotensin directly liberate catecholamines from the adrenal glands of the Guinea-pig, as in other mammals, and that each thereby reduces its own bronchoconstrictor

action. Vogt (1965) perfused the isolated adrenal gland of a dog with Locke's solution and concluded that the receptors sensitive to acetylcholine and to potassium chloride survive well, whereas those interacting with angiotensin, bradykinin and histamine are readily disrupted. Both angiotensin 8μgm and bradykinin 20·0μ gm produced a small release of medullary amines, and the doses required were much higher than *in vivo*.

Robinson (1967) also studied the catecholamine output of the isolated, perfused adrenal gland of the dog by angiotensin and bradykinin. He found that both angiotensin and bradykinin were extremely potent liberators of adrenal catecholamines. He confirmed the observation of Feldberg and Lewis (1964) and estimated from their data that one molecule of bradykinin released at least 50 molecules of adrenaline and one molecule of angiotensin released at least several thousand molecules of adrenaline from the adrenal medulla in the cat.

Robinson did not agree however with the finding of Vogt (1965) that the isolated, perfused adrenal gland of the dog is relatively insensitive to angiotensin and bradykinin owing to damage of their respective receptors by perfusion with Locke's solution. His results were consistant with the hypothesis proposed by Douglas and Rubin (1963) that calcium acts as a link between the stimulus and the secretory mechanisms in the chromaffin cells.

An attempt has been made to show *in vivo* angiotensin

stimulation of the adrenal medulla by analysing plasma catecholamine concentrations in blood samples drawn from the inferior vena cava of anaesthetized dogs (Peach, Cline and Watts, 1966). It was found that angiotensin produced an increase in circulating catecholamines. Adrenaline was significantly increased by angiotensin infusions of 0·05 and 0·1 μg/kg/min. Plasma noradrenaline was significantly increased also except with a 0·5 μgm/kg injected dose of angiotensin. They observed prolonged adrenaline response which could be due to the continued stimulation of the adrenals by the relatively large amounts of angiotensin accumulated in the gland.

In the Guinea-pig, the intravenous dose of bradykinin, 0·75 μgm; histamine, 4·4 μgm; and slow reacting substances in anaphylaxis, 1·5 mg, caused a relaxation of superfused rat stomach strip and chick rectum equivalent to that caused by an intravenous dose of 0·1 μgm of adrenaline (Piper, Collier and Vane, 1967). The liberation of the adrenaline by these substances could arise from a direct or an indirect stimulation of the adrenal medulla. Hexamethonium failed to block the release of adrenaline by bradykinin suggesting that the stimulation was not transmitted through a normal ganglionic pathway.

Angiotensin was about twice as potent as bradykinin (Piper and Vane, 1967) the equivalent dose to that caused by an intravenous dose of 0·1 μg adrenaline being 0·37 μg angiotensin which had about the same potency when injected intra-arterially. The release of adrenaline

by angiotensin was not blocked by hexamethonium, suggesting that angiotensin acted directly on the adrenal medulla. Staszewska-Barczak and Vane (1967) studied the release of catecholamine from the adrenal medulla by peptide in the dogs and cats using blood-bathed stomach strip and chick rectum to detect the relative concentrations of catecholamines released. They believed that the catecholamine released was mainly if not entirely adrenaline, the concentration depending on the dose of the peptide used. No release was observed after adrenalectomy. Clearly wide species differences exist. The effect of angiotensin on the bovine adrenal medulla has also been studied (Comline, Silver and Sinclair, 1968). These workers found that angiotensin had very little effect either directly or indirectly on the secretory response of adrenal medulla in this animal.

Renin-Angiotensin Release by Catecholamine.

A possibility of renin-angiotensin release by catecholamines still exists. In anaesthetized dogs renin release was studied during the infusion of catecholamine by Wathen, Kingsbury, Stouder, Schneider and Rostorfer (1965). They showed a marked increase in renal vein renin occurred within 1 minute of the onset of catecholamine infusion into the renal artery and continued for 15 minutes beyond the infusion period. During intravenous infusion of noradrenaline, renin release did not usually occur but on stopping the intravenous

infusion the ensuing fall in blood pressure was coincident with a marked release of renin. Bunag, Page and McCubbin (1966) found that neural stimuli in anaesthetised dogs are capable of causing a release of renin in the absence of gross changes in renal perfusion pressure or flow. They also showed that stimulation of the sympathetic vasomotor discharge by occlusion of the common carotid arteries, while renal perfusion pressure was kept constant, also caused release of renin, as did infusions of noradrenaline, tyramine or DMPP. In 1967, Gordon, Otokuchel, Liddle and Island showed that plasma renin activity of a normal subject increased in response to the infusion of catecholamines (noradrenaline and adrenaline) and increased with stimulation of the sympathetic nervous system by cold. They suggested a direct effect of catecholamines on renal receptors, and noradrenaline infusion can restore a blunted renin response in humans. Johnson, Davis and Witty (1971) suggested that adrenaline increased renin secretion in the nonfiltering kidney by an action on the renal arterioles while noradrenaline and renal nerve stimulation apparently increased renin secretion in the nonfiltering kidney by a direct effect on the juxtaglomerular cells.

In further support of a possible role for catecholamine in releasing renin are the studies describing direct stimulation <u>in vitro</u> of renin production by the catecholamine hormones (Michelakis, Caudle and Little, 1969). They prepared a dog renal suspension suitable for the study of renin

production by processing a dog kidney. The addition of adrenaline, noradrenaline, or cyclic AMP to the incubated control suspension caused striking increases in net production of renin. This renin production was abolished by cycloheximide, an inhibitor of protein synthesis.

METHODS

Animals:

New Zealand white rabbits of either sex were used in these experiments, the body weight ranging from 1·9 - 3·5 kg.

Dogs of either sex were used, the body weight ranging from 9 - 25·5 kg.

Preparation of Animals.

1 - Rabbits: The rabbits were anaesthetized with sodium pentobarbital 40 mg/kg body weight diluted with normal saline 1:1 (volume/volume) and injected very slowly through the ear vein. Further doses of anaesthetic were given intravenously when necessary during the course of the experiment.

In some of the early experiments the anaesthesia was carried out in two stages. An initial dose of mixture of 24 mg/kg body weight sodium pentobarbital with 0·5 mg/kg body weight chloralose (2·5%) / urethane (25%) injected through an ear vein. After half an hour a second dose of 24 mg/kg body weight sodium pentobarbital was injected by the same way. Further doses of anaesthetic were given intravenously when necessary.

After induction of anaesthesia the animal was placed on its back, the body temperature was maintained by using a homeothermic blanket (Electro-Physiological Instruments Ltd.) under the animal and thermocouples were used to

record forelimb skin and rectal temperature. The neck was shaved and a midline incision 4 - 5 cm long made. The trachea was exposed and dissected free. Ties were passed around the trachea, which was then opened with a transverse cut between two tracheal rings and a polythene cannula of suitable size inserted and tied in place to maintain the airway.

One carotid artery was cannulated with a polythene cannula (0·9 mm internal diameter). This was connected to the blood pressure transducer. The right external jugular vein was cannulated with a polythene cannula (1·52 mm internal diameter). This formed the return tube for the extracorporal circuit.

The groin area on both hind legs was shaved and a 4 - 5 cm long incision was made on each leg just above the femoral blood vessels. The femoral artery and vein were dissected free and polythene tubes (1·19 mm internal diameter) inserted in the right femoral artery and vein. The venous cannula was used for injection and transfusion and the arterial cannula for bleeding and blood sampling.

A polythene tube about 45 cm long and 1·19 mm internal diameter was used to cannulate the left femoral artery and vein. The right kidney (the more cranial one) was palpated from outside very easily just below the last rib. The distance from the cranial end of the kidney along the midline of the abdomen following up the line of the inferior vena cava and aorta, femoral artery and vein to the point of cannulation was measured with the

cannulae which were then marked so that they could be inserted to the appropriate level. One of the tubes was inserted into the femoral vein and carefully pushed into the vessel up to the mark. The tip of the cannula then lay just cranial to the right renal vein and the entry of the adrenal out flows. In some early experiments the cannula was introduced via the right femoral vein and the cannula disappeared into side branches from the right side of the vena cava supplying the mesentary and body wall. This difficulty did not arise when the cannula was introduced from the left side. Position of the cannula was checked at the end of the experiments by laparotomy. Using the same technique the other long cannula was inserted into the left femoral artery to the abdominal aorta until it reached the mark which indicated that the tip of the cannula was just cranial to the right renal artery. The venous tube forms the outflow from the vena cava for the extra corporeal circuit and the arterial tube enabled intra arterial infusions close to the adrenal glands to be given.

In a group of three rabbits a midline incision was made in the abdomen, both adrenal glands were located and the adrenal veins were dissected free. A fine thread was passed around each vessel to enable them to be tied off later in the experiment. In some other experiments the renal artery and vein were dissected free and ties passed around them to enable them to be tied off later in the experiment.

2 - Dogs: The dogs were anaesthetized by sodium pentobarbital 42 mg/kg body weight slowly into the brachial vein. In two early experiments gas anaesthesia was used. After an initial dose of thiopentone sodium 20 mg/kg body weight, a mixture of halothane/O_2/N_2O was used until the surgical operation was finished. A dose of 70 mg/kg body weight chloralose 1% solution, was then given intravenously to maintain the anaesthesia during the remainder of the experiment. Further doses of chloralose were given intravenously when necessary.

The same surgical procedure was carried out to cannulate the same vessels as in the rabbits, the only difference being the calibre of the cannulae and their length. For the carotid artery a 1·49 mm internal diameter cannula was used, jugular vein cannula 1·4 mm internal diameter, right femoral artery and vein 2·0 mm internal diameter, and left femoral artery and vein a 50 cm long and 1·90 mm internal diameter polythene tubes were used throughout.

A metal cannula with sidearm was inserted into the trachea and tied in place with string.

In groups of three dogs a long incision was made in the midline of the abdomen and the renal vein on each side located and dissected free. A fine thread was passed around the vein using an aneurysm needle. This thread was used later on to prevent venous outflow from the kidney reaching the vena cava.

All animals, rabbits and dogs, were allowed to breath

normal air spontaneously during the initial stages of the experiment until the surgery was completed. The animal was then allowed to recover from the surgical techniques for 30 - 60 minutes depending on the severity of the surgery involved. Just before the extracorporeal circuit was connected up the animals were switched to breath a 50% oxygen mixture in air through non return valves of the Reuben type for dogs and a small home made variant for the rabbits.

The Superfusion Circuit.

This involved the continuous withdrawal of venous blood from the inferior vena cava just above the level of the right adrenal gland to superfuse a smooth muscle strip continuously and return back to the animal again via the jugular vein cannula. It was thought that this blood would contain a large proportion of the released adrenal medullary hormones from both glands. The blood would also contain some proportion of the angiotensin generated by the release of renin from the kidneys.

For the purpose of superfusion a roller pump with controllable speed (Watson-Marlow Ltd., England) was used. Soft silicon rubber tubes (2 mm internal diameter) were used to carry the venous blood from the animal, through the roller pump to a heating jacket and superfusion chamber. Return flow was from the superfusion chamber via the pump to be returned to the animal The extracorporeal circuits were washed through with

Diagram 1.

The extracorporeal superfusion circuit. The tip of the left femoral vein cannula (4) was pushed to lie in the inferior vena cava just central to the venous outflow of the right kidney (1), vena caval blood was withdrawn in the input tube (7) by the roller pump (12), the blood was kept warm in a heated bath at 41-42^{o}C (13) and then dripped on a rat stomach strip (15) in a superfusion chamber (14) and passed back into a return flow silicon tube (8) via the pump (12) then returned to the animal via the jugular vein cannula (10).

The tension of the muscle strip is monitored by using a stain gauge (16) and recorded on a 4-channel Devices M4 or MX4 recorder (17). The arterial blood pressure was recorded from the carotid artery (9) using a Devices pressure transducer (11) and displayed on the 4-channel Devices recorder (17).

Infusion of angiotensin II was carried out by a cannula (3) inserted through the femoral artery and passed through the abdominal aorta until the tip lay central to the right renal artery.

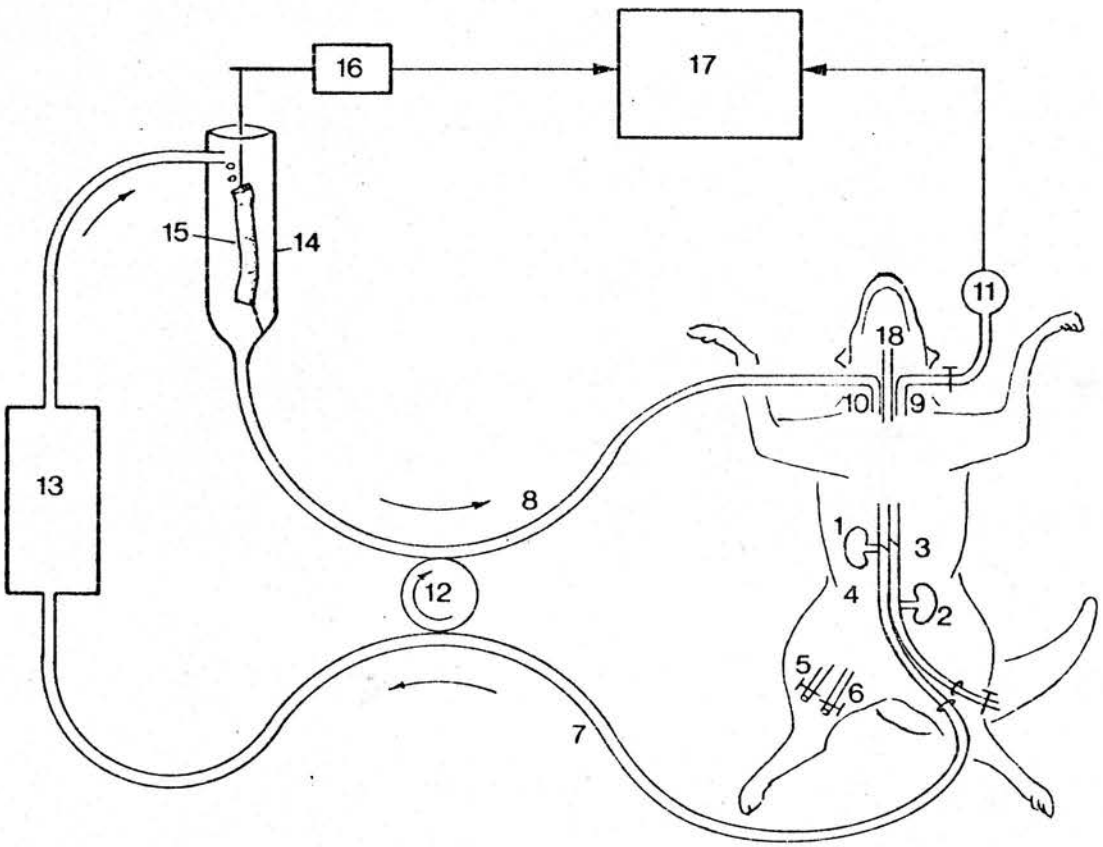
Injection of anaesthetic was carried out by right femoral vein cannula (6).

Bleed commenced by a cannula (5) which was inserted into the right femoral artery.

(18) a tracheal cannula.

(2) the left kidney.

Extracorporeal superfusion circuit



saline before the experiment and filled with saline or dextran-saline just before connection to the preparation. The animal was heparinized and the extracorporeal input tube connected to the left femoral vein cannula. The blood passed through the pump and water from a small thermostatically controlled heated bath was circulated continuously by pump through the water jacket. A temperature of 41 - 42°C in the bath ensured the blood temperature being at 37 - 38°C as it passed on to the superfused muscle strip. The tube from the water jacket passed into the superfusion chamber to allow a continuous flow over a strip suspended in the chamber. The chamber itself was made of perspex (8 cm long, 1·5 cm internal diameter and 0·3 cm thick) fitted with a cap through which a thread from the muscle strip could be attached to a strain gauge and the stainless steel rod and hook for anchoring the muscle strip could be passed.

Blood flows in the external circuit were adjusted at 10 ml/min for rabbits and 15 ml/min for dogs.

Blood from the base of the perspex chamber returned to the animals jugular vein via the roller pump (Diagram 1).

Muscle Strip Preparation.

Biological assay techniques were used in these experiments. The rat fundus (stomach) strip (Armitage and Vane, 1964) was used for catecholamines and the rat ascending colon strip (Regoli and Vane, 1964a) for

determining changes in angiotensin levels.

Early experiments gave very variable sensitivities for the fundus strip. It was noticed that female rats often gave a more sensitive preparation than male rats. It was thought that this might be related to the level of reproductive hormones particularly oestrogens. Haigh, Kitchin and Pickford (1963) have shown that oestrogens enhance the vasoconstrictor effects in vascular smooth muscle and it seemed possible that might enhance the inherent tone of the fundic smooth muscle and thus increase the subsequent effects of catecholamines in reducing the muscle tone. This proved to be the case, and in all subsequent experiments female rats of average weight between 200 - 250 gm were used. An oestrogen, (stilboestrol diproprionate 0·1 mg/kg body weight) was injected subcutaneously 18 hours before the experiment.

The rats were killed by a blow on the head followed by slitting the throat. The abdomen was opened and the stomach and ascending colon removed into a dish of Krebs solution containing (g/l of distilled water): NaCl 6·9; KCl 0·35; $CaCl_2$ $6H_2O$ 0·55; KH_2PO_4 0·16; $MgSO_4$ $7H_2O$ 0·29; glucose 1; and $NaHCO_3$ 2·1.

The fundus part of the stomach is easily distinguished by its transluscent balloon-like tissue and was separated from the pyloric part which is thicker and redder.

Fundus and ascending colon were opened longitudinally using round ended scissors to avoid tissue damage. The tissues were carefully washed clean of their contents

with fresh clean Krebs solution in a beaker. Thin longitudinal strips 4 - 5 cm long were cut from the fundus and one suspended in the superfusion chamber from a supporting hook. A thread from the free end of the strip was connected to a strain gauge (Devices STD 2 - 100 gm range). With the gut strip suspended in the middle of the chamber blood was allowed to pass from the input tube down the suspending strip and on to the preparation in a continuous flow. The remaining strips were stored in Krebs solution in a refrigerator and could be used if the initial preparation was insensitive.

When observing changes in angiotensin levels a strip of ascending colon 4 - 5 cm long was used. This was soaked in a β - blocker propranolol, (10^{-6} g/l solution) for half an hour to block any inhibitory effect of catecholamines. The strip was then suspended in the chamber as described for fundus.

Even though the biological assay techniques are not accurate nor very specific they alone can monitor small continuous changes in the circulating levels of catecholamines or angiotensin. While biochemical methods of estimation would have given more precise results in terms of hormone concentrations, it would have been impossible to take the very large number of blood samples required to build up a similar picture of responses. Particularly in the rabbits, the loss of blood involved would have nullified the experiment. Rapid or transitory changes in hormone concentrations would certainly have been missed.

For specificity of the stomach strip assay see the appendix on P.174.

Calibration of the Superfused Tissue.

The tissues were roughly calibrated by intravenous injection of adrenaline or angiotensin into the right femoral vein cannula. After injection of known doses of adrenaline or angiotensin the relaxation of the rat fundus strip as response to adrenaline or the contraction of the rat ascending colon as a response to angiotensin was recorded. Changes of tension were measured in centimeters. The changes in tension were calculated as a percentage of resting tension.

The transducer was calibrated, using the same recording parameters, by hanging known weights from the strain beam. Since it is impossible to assess the percentage of the inferior vena caval flow passing to the strip a few experiments were carried out in which known doses of catecholamines were introduced directly into the flow input tube through a side arm. This indicated a roughly 1:10 dilution of calibrating doses injected into the venous circulation. In the rabbit the delay between i/v injection of a test dose and response of the stomach strip was about 60 seconds. In dogs where faster flows were employed it was about 40 seconds.

Experimental Procedures.

1 - Haemorrhage. In rabbits an infusion/withdrawal pump model 600 - 900 (Harvard Apparatus Co. Inc.) was used. Two 30 ml siliconed glass syringes were used in the pump and blood was withdrawn from the right femoral artery cannula at a rate of 14 ml/min. Haemorrhage was discontinued when the mean arterial blood pressure reached about 60 mmHg. The removed blood was kept in a water

bath at 37°C for periods of up to 30 minutes after which it was re-transfused using the pump at a rate of 8 ml/min.

In dogs the technique differed slightly, the blood being allowed to flow at a restricted rate of about 40 ml/min from the right femoral artery. The blood passed from the 3-way tap of the cannula through a siliconed rubber tube to be collected in a siliconed glass measuring cylinder. After the haemorrhage was complete the blood was transferred to a transfusion bottle, sealed, and kept in the water bath at 37°C. After periods of hypotension lasting up to 30 mins the blood was returned slowly by an intravenous drip through the right femoral vein cannula.

2 - Administration of Drugs.

Muscarinic blockade: To block the muscarinic receptors (-) hyocine methyl bromide (Scopolamine methyl bromide) was infused intravenously at 5 mg/kg body weight by slow infusion (1·53 ml/min) using the Harvard infusion/withdrawal pumps. After infusion at least 5 - 10 minutes were allowed for the animal to settle down before haemorrhage was repeated.

Nicotinic blockade: To block the nicotinic receptor sites hexamethonium bromide was given intravenously at 10 mg/kg body weight slowly (1·53 ml/min) using the Harvard infusion/withdrawal pump.

After the infusion of blocking agents arterial blood pressure sometimes fell to unacceptable levels. In

these experiments a dextran-40 drip was given and the blood pressure stabilized at an acceptable level before haemorrhage procedures were repeated.

Angiotensin: Angiotensin was infused intra-arterially through the long left femoral artery cannula at 100 ng/kg/min and increased gradually to 400 ng/kg/min for a period of 10 minutes. These infusions were made using the slow infusion apparatus (C.F. Palmer & Co.).

Injection of anaesthetic, heparin, and test doses of adrenaline and angiotensin were given intravenously through the right femoral vein cannula. Retransfusion of the shed blood and infusion of blocking agents were also made through this cannula.

Drugs:

Sodium pentobarbitone (Nembutal, Abbott, 60 mg/ml).

Heparin (Pularin, Evans Medical Ltd., 25,000 IU/5 ml) was administered intravenously in a dose of 1000 IU/kg body weight for both species.

Adrenaline tartrate (Evans Medical Ltd.) 1 in 1000. Test solutions (0·5 μgm/ml in 0·9% saline) were used for testing the sensitivity of the fundus muscle strip. This solution was stored in the refrigerator between use.

Angiotensin (asparaginyl amide, Hypertensin, Ciba) was prepared in 0·9% saline in a concentration of 125 ng/ml for testing responses of the ascending colon. Test doses of 1 ml were used for both drugs.

Carbachol B.P. (The British Drug Houses Ltd.) was prepared in 0·9% saline in a concentration of 1 mg/ml.

Dextran-40 injection B.P. 10% in 0·9% sodium chloride (Lomodex 40, Fisons Ltd.).

Scopolamine methyl bromide (Sigma Chemical Co.). Given intravenously in doses of 5 mg/kg body weight in saline.

Hexamethonium bromide (Sigma Chemical Co.). Given intravenously in doses of 10 mg/kg body weight in saline.

Stilbestrol Dipropionate (Burroughs Wellcome & Co.) 10 mg/ml was diluted in olive oil and used in a dose of 0·1 mg/kg body weight subcutaneously.

Recording System.

To record the results a four channel Devices M4 or MX4 recorder was used. Heart rate was recorded on the first channel using a ratemeter. Needle electrodes piercing the skin of the four limbs but not penetrating the underlying muscle were applied and the four ECG leads connected to an ECG amplifier. The output was passed to the ratemeter which was triggered from the QRS complex. Two types of ratemeter were used. In the Devices ratemeter, change of rate was indicated as a result of change of interspike interval which is converted to give an output voltage proportional to the reciprocal of time. A change in voltage represents a change in interval of the two previous pulses. With this ratemeter the input signal (ECG) could be monitored in preference to the rate signal when required on the same pen channel using the MX4 recorder. In the second type of

ratemeter the input signal triggered a small increase in voltage at each pulse giving a stepwise increase in voltage. This was returned to baseline at preset intervals by a time circuit. In some of the early experiments the heart rate was measured by running the chart on high speed and counting the heart beats per minute from the blood pressure pulse wave. In a small number of experiments the blood pressure pulse was used instead of the ECG wave to trigger the ratemeter.

The tension of the rat stomach strip was continuously monitored using a (Devices STD2-100 gm range) strain gauge, its output being connected to a bridge unit and high gain D.C. amplifier and displayed on the second channel of the recorder.

Arterial blood pressure was continuously monitored on the third channel using a pressure transducer (Devices CEC) type 4-327-L221. A mercury manometer could be connected in series for calibration checks when required. The transducer was connected through a plastic 3-way tap and fine cannula to the carotid artery.

In some of the early experiments the central venous pressure was recorded on one of the channels in the same manner used in recording the arterial blood pressure except for the higher sensitivity, venous blood pressure being recorded over a 40 mmHg range compared with the 250 mmHg range used for arterial pressure.

Respiratory tidal volume was recorded on the fourth channel using the F2 6 mm 0·525 flow head (Mercury

Electronics) for rabbits and F2 19 mm 0·20 flow head for dogs, flow head being connected to electrospirometer CS5 (Mercury Electronics). When air passes through the flow head, there is a small change in pressure across the gauze resistance which is linearly related to velocity flow over a specified range. The pressure transducer generates an electrical signal proportional to the pressure changes produced in the flow head, and when the signal is integrated with respect to time, it can be calibrated in terms of volume flow. At the beginning of each inspiration or expiration a threshold switch connects the manometer output to the input of the integrator which can be used in two ways.

The first is in recording tidal volume. At the beginning of inspiration the threshold switch connects the manometer to the integrator and inspiratory volume is recorded. At the end of inspiration it resets the output zero where it remains until the next inspiration begins. Thus the output on a pen recorder will appear as a histogram of tidal volumes.

A second method of recording used occasionally was cumulative recording of minute volume. In this mode each tidal volume was recorded as a small stepwise increase in zero being reset at appropriate intervals by a timing circuit.

Measurements of blood pH, pCO_2 and pO_2 were carried out using the IL 213 blood gas analyser (Instrumentation Laboratory Inc.). This consists of a sensitive electro-

meter with digital read out, and a temperature controller with a capability of $\pm$ 0·5°C.

The pH system consists of a glass micro-electrode requiring only 0·1 ml blood sample, and a calemel reference electrode. It operates conventionally, the potential difference being proportional to the number of hydrogen ions present. Calibration is carried out using the "balance" control to set the zero of the instrument to that of a buffer of pH 6·84. The gain of the amplifier is adjusted by means of the "slope" control to the value of a buffer of pH 7·384. This ensures accuracy around the pH found in blood.

The CO_2 system is a pH sensitive glass electrode which measures the pH of a film of sodium bicarbonate solution. Equilibration of the solution with CO_2 in blood occurs across a polytetrafluoroethylene (Teflon) membrane which is readily permeable to CO_2, but not to other ions which might alter the pH of the bicarbonate solution.

The oxygen system follows the design of Clark (1956), and is a polarographic method. The electrode produces an electric current at a constant polarising voltage (0·6 V) which is directly proportional to the PO_2 diffusing to the reactive surface of the electrode. The current is the result of the reduction of oxygen at the platinum cathode as follows:-

$$O_2 + 2H_2O + 4e^- \longrightarrow 4OH^-$$

Each molecule of oxygen reacts with four electrons and the electron flow is measured. The electrons are provided by silver-silver chloride anode, where silver is oxidised:-

$$Ag + Cl^- \longrightarrow AgCl + e^-$$

Response time is two minutes to reach 95% of final value. Calibration of both oxygen and carbon dioxide electrodes is carried out using two known gas mixtures metered by a volumetric pump (IL.308). The "low" gas which contains 5% CO_2 and 10·5% O_2 is used with the "balance" controls to set the zero position for CO_2 and the "slope" for O_2. The "high" gas which contains 10% CO_2 and 0% O_2 sets the zero position for oxygen and the amplifier gain for CO_2 using the "slope" controls. An 0·5 ml blood sample was withdrawn in a clean 1 ml glass syringe after first withdrawing a rather larger blood sample to clear the cannula dead space. In rabbits the blood was recovered after the measurements and returned to the animal with the "dead space" sample.

In some early experiments a check on the percentage haemoglobin concentration was made using the cyanmethaemoglobin method with a Corning-EEL Model 197 Spectra Colorimeter.

Packed cell volume was checked from time to time using the Hawksley micro-haematocrit.

RESULTS

Circulatory and Respiratory Responses to Haemorrhage.

As a preliminary to the main part of the investigation the responses of rabbits and dogs to a standard haemorrhage procedure were compared.

As mentioned earlier in the methods, the animals were bled slowly from a femoral arterial cannula until a mean blood pressure of about 50 - 60 mmHg was obtained. In rabbits the rate of bleed was exactly controlled using siliconed syringes on infusion/withdrawal pumps (Harvard Co.). In dogs, the rate of bleed was less precisely controlled using a screw clip on the tube connected to an arterial cannula. The hypotensive situation was maintained, apart from the animals own compensatory adjustments for a minimum of 30 minutes in most experiments unless the condition of the animal precluded it. The removed blood was collected in silicon or plastic vessels and placed in a water bath, at 37^{o}C until re-infused.

Return of blood was achieved via the femoral vein with the infusion pump in the rabbits and by gravity drip in the dogs.

1. The Circulatory Responses following Haemorrhage.

a) Rabbits.

The results of all the experiments indicate that heart rate increased quantitatively during the first

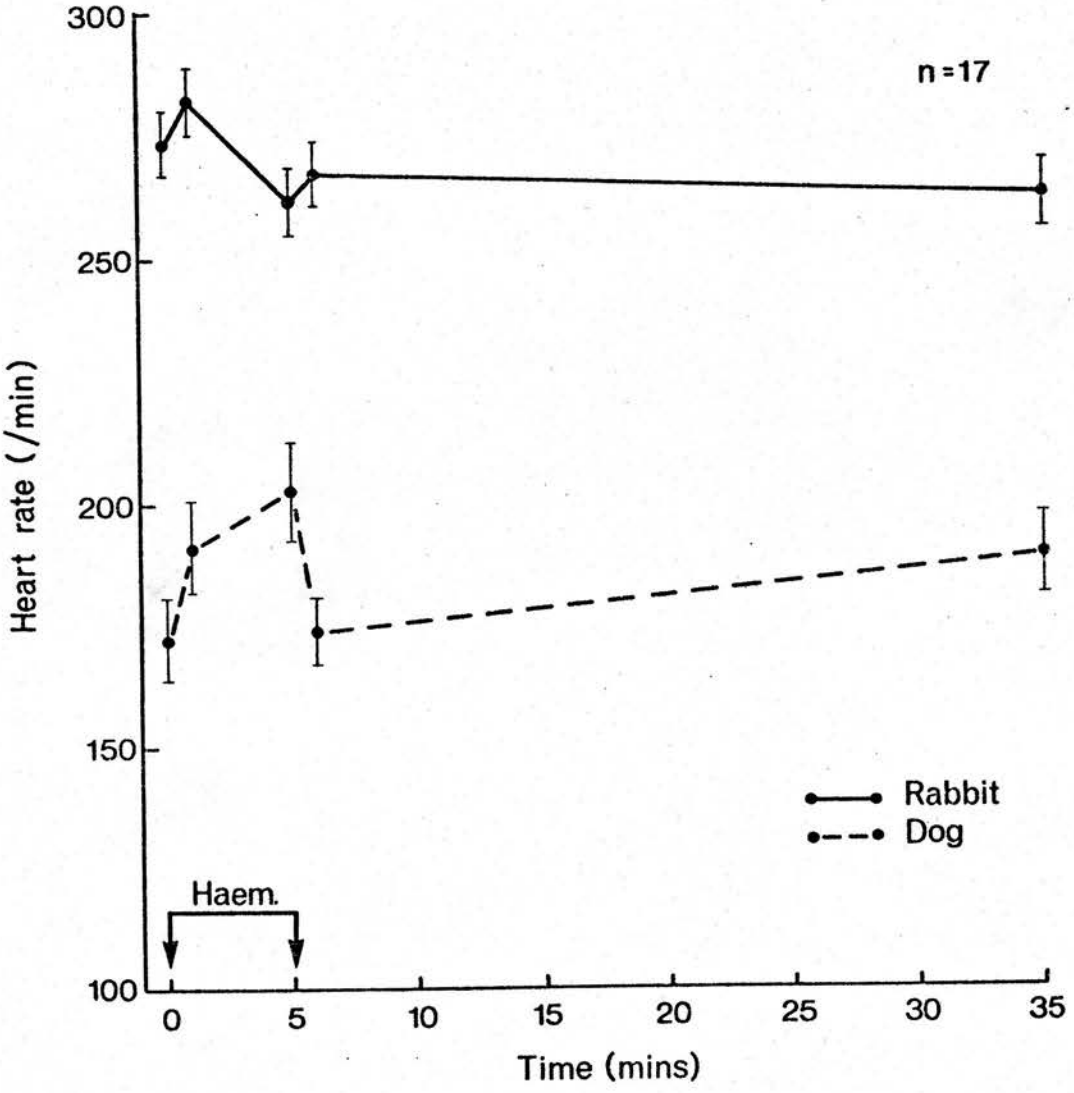

300
250
200
150
100
n=17
Heart rate (/min)
Rabbit
Dog
Haem.
0
5
10
15
20
25
30
35
Time (mins)

TABLE 1

Before Haemorrhage	During Haemorrhage		After Haemorrhage	
Control	1st min	End of bleed	1 min	30 min
Beats/min	Beats/min	Beats/min	Beats/min	Beats/min
305	305	290	290	280
290	305	280	290	280
260	270	230	320	210
280	270	220	240	290
290	290	230	240	290
220	225	210	210	220
290	290	290	285	270
270	270	260	260	250
260	290	270	270	260
250	260	260	265	270
280	300	290	280	270
270	280	270	270	290
230	240	230	230	200
318	330	312	300	300
250	280	280	280	260
280	260	255	250	260
310	330	270	270	270
273·7 ± 6·5	282·1 ± 6·8	261·6 ± 7·0	267·7 ± 6·7	262·9 ± 7·0

The total at the foot of each column represents Mean ± S.E.

Table 1. The heart rate (beat/min) before, during 1st min, at the end of haemorrhage, after 1 min, and after 30 minutes of haemorrhage in 17 rabbits.

minute of bleed but only by 8·3 ± 9·7 (S.E. of difference) beats/min. This change was not significant ($P < 0.4$), neither was the blood pressure significantly reduced at this stage.

At the end of the haemorrhage, which took almost 5 minutes to complete, the mean arterial blood pressure was significantly reduced 28·0 ± 4·2 (S.E. of difference) mmHg ($P < 0.001$) the heart rate also decreased but not significantly 11·12 ± 9·54 (S.E. of difference) beats/min. and continued at this level until 3 - 4 minutes after completion of the haemorrhage when it started to rise again. But even when internal compensation had raised the blood pressure (11·25 ± 4·06 (S.E. of difference) mmHg) 30 minutes after the end of the bleed the heart rate remained far below that of the control (Table 1 and Fig 1).

Similar changes in heart rate and arterial blood pressure were observed during haemorrhage carried out after muscarinic blockade administered to the animal. After the administration of nicotinic blockade the blood pressure fell sharply and significantly at the end of the bleed but was not accompanied by significant increase in heart rate.

b) Dogs.

In these experiments some major differences were noted compared to the rabbits. The heart rate was slightly increased 18·75 ± 13·1 (S.E. of difference) beats/min during the first minute of haemorrhage with decrease in arterial blood pressure. At the end of bleed,

TABLE 2

Before Haemorrhage	During Haemorrhage		After Haemorrhage	
Control	1st min	End of bleed	1 min	30 min
Beats/min	Beats/min	Beats/min	Beats/min	Beats/min
119	132	140	140	142
182	198	192	180	210
170	188	215	186	222
190	205	220	198	193
180	192	198	160	185
175	215	225	180	180
200	220	240	175	200
160	176	192	172	180
$172 \pm 8{\cdot}7$	$190{\cdot}8 \pm 9{\cdot}8$	$202{\cdot}8 \pm 10{\cdot}8^{*}$	$173{\cdot}9 \pm 6{\cdot}2$	$189{\cdot}0 \pm 8{\cdot}5$

The total at the foot of each column represents Mean $\pm$ S.E.

* $P < 0{\cdot}05$.

Table 2. The heart rate (beat/min) before, during 1st min, at the end of haemorrhage, after 1 min, and after 30 minutes of haemorrhage in 8 dogs.

which lasted about 5 - 8 minutes, the heart rate continued increasing to reach a maximum of 12·00 ± 14·57 (S.E. of difference) beats/min at the end of bleed, this increase being statistically significant ($P < 0{\cdot}05$). This was accompanied by a significant decline in arterial blood pressure. Just after the haemorrhage was stopped heart rate started to fall and after one minute it reached its lowest rate for the whole haemorrhage period although still slightly above normal. Later it began to rise again but this increase was not significant. The mean arterial blood pressure rose 17·5 ± 5·8 (S.E. of difference) mmHg during the 30 minutes following the end of haemorrhage (Table 2, Fig 1).

Similar changes in heart rate and arterial blood pressure were observed during haemorrhage carried out after muscarinic blockade administered to the animal. However, after administration of nicotinic blockade the blood pressure declined sharply and significantly at the end of the bleed but was not accompanied by significant increase in heart rate.

Effect of Haemorrhage on Blood Dilution by Fluid Transfer.

Arterial blood samples were taken for haematocrit and haemoglobin concentration determinations in 12 rabbits and 6 dogs before, immediately after and 30 minutes after the end of haemorrhage. Analysis of observations reveals a quite interesting difference between the two species of animals.

Figure 2.

The left panel represents the mean values of the blood (p.c.v.) packed cell volume % (upper) and haemoglobin (Hb.) concentration g/100 ml (lower) of 6 dogs.
The right panel represents the mean values of the blood (p.c.v.) packed cell volume % (upper) and haemoglobin (Hb.) concentration g/100 ml (lower) of 6 rabbits.
The arrows point to the times of start and finish of haemorrhage.

Bars represent standard errors.

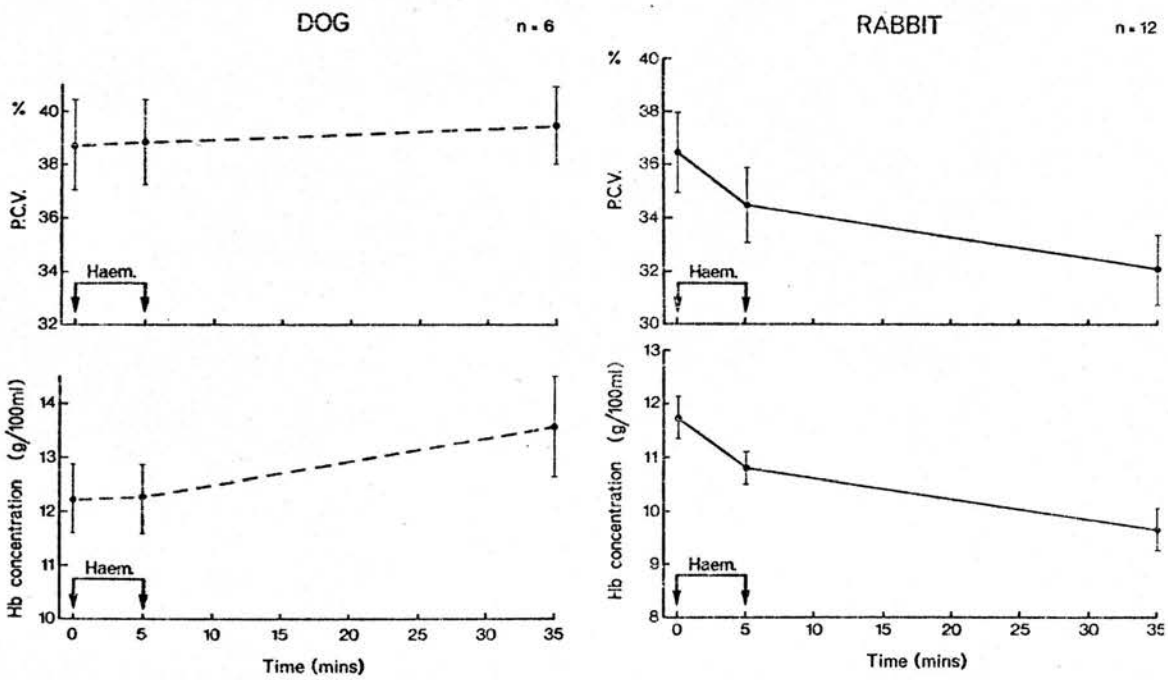

DOG
n = 6
RABBIT
n = 12
%
P.C.V.
Haem.
Hb concentration (g/100ml)
Time (mins)

TABLE 3

Before Bleed		End of Bleed		30 min after Bleed	
P.C.V.	Hb	P.C.V.	Hb	P.C.V.	Hb
37·00	12·929	37.50	11·374	38·50	9·303
40·75	11·981	38·00	11·130	37·00	10·628
38·00	12·665	33·00	11·358	35·75	10·786
38·10	12·344	37·30	11·448	30·90	10·267
37·25	11·563	33·50	10·419	32·00	9·111
35·00	10·592	37·50	10·630	33·50	10·176
23·00	8·131	24·00	8·812	24·50	6·929
34·50	11·360	33·00	10·51	31·50	10·150
38·50	12·398	33·50	10·828	32·00	9·133
32·50	11·032	28·00	9·264	23·00	7·150
41·50	13·942	40·75	12·075	32·75	10·605
41·20	12·135	40·75	11·826	33·50	11·092
36·44 $\pm$ 1·45	11·756 $\pm$ 0·419	34·73 $\pm$ 1·44	10·806 $\pm$ 0·281	32·08 $\pm$ 1·31*	9·641 $\pm$ 0·399**

The total at the foot of each column represents Mean $\pm$ S.E.

* $P < 0{\cdot}05$. ** $P < 0{\cdot}01$

Table 3. The packed cell volume (P.C.V.) % and Haemoglobin concentration (Hb) gm/100 ml of arterial blood samples of 12 rabbits before, at the end and 30 minutes after the haemorrhage.

TABLE 4

Normal		End of Bleed		30 min after Bleed	
P.C.V.	Hb	P.C.V.	Hb	P.C.V.	Hb
36·0	13·10	36·3	13·20	38·2	13·60
46·5	10·20	46·0	10·26	46·0	12·65
37·0	12·00	36·0	12·22	37·2	12·70
35·3	14·80	36·0	14·80	36·0	18·20
40·5	11·80	40·8	11·96	40·9	12·63
37·0	11·42	38·0	11·20	38·2	11·81
38·7 $\pm$ 1·7	12·22 $\pm$ 0·64	38·9 $\pm$ 1·6	12·77 $\pm$ 0·65	39·4 $\pm$ 1·5	13·60 $\pm$ 0·95

The total at the foot of each column represents Mean $\pm$ S.E.

Table 4. The packed cell volume (P.C.V.) % and Haemoglobin concentration (Hb) gm/100 ml of arterial blood samples of 6 dogs before, at the end, and 30 minutes after the haemorrhage.

a) Rabbits.

In twelve observations almost all show dilution of blood indicated by the decrease of both packed cell volume and haemoglobin concentration occurring after the end of haemorrhage. Dilution shown at the end of bleed, approximately 5 minutes after the onset of the haemorrhage was unexpected. Thirty minutes after the end of haemorrhage blood dilution was significant, haemoglobin concentration being decreased 2·11 $\pm$ 0·57 (S.E. of difference) g/100 ml from that of the control ($P < 0{\cdot}01$). Packed cell volume was also decreased 4·36 $\pm$ 1·95 (S.E. of difference)% from the control ($P < 0{\cdot}05$) (Table 3, Fig 2).

b) Dogs.

In six observations results show a difference from those in the rabbits. The vascular system of the dogs is alleged to be very sensitive to changes in hydrostatic and onchotic pressure allowing fast shifts of fluid to or from the blood.

No dilution in the blood was apparent either at the end of the bleed or after 30 minutes of recovery. In fact at the end of haemorrhage a slight increase in haemoglobin concentration and packed cell volume was noted (Table 4 and Fig 2).

In unpublished observation from class experiments in the department, dilution of blood is noted 60 minutes after the end of haemorrhage.

Figure 3.

The left panel represents the mean values of the arterial pH (upper), arterial pCO_2 (p_aCO_2) in the middle, and the arterial pO_2 (p_aO_2), the lower, of 6 dogs.

The right panel represents the mean values of the arterial pH (upper), arterial pCO_2 (p_aCO_2) in the middle, and the arterial pO_2 (p_aO_2), the lower, of 6 rabbits.

The arrows point to the time of start and finish of haemorrhage.

Bars represent standard errors.

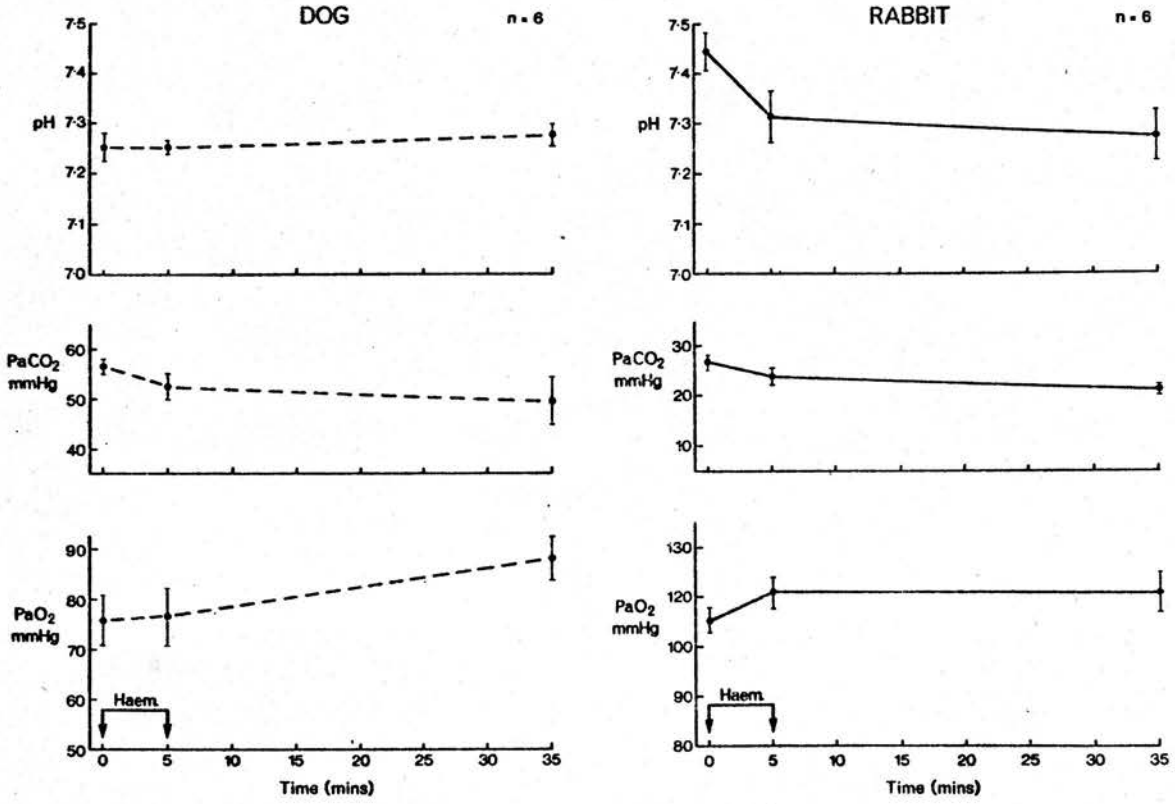

DOG
n = 6
RABBIT
n = 6
pH
PaCO2 mmHg
PaO2 mmHg
Haem.
Time (mins)

TABLE 5

Before Bleed			End of Bleed			30 min After Bleed		
p_aCO_2	p_aO_2	pH	p_aCO_2	p_aO_2	pH	p_aCO_2	p_aO_2	pH
25·6	102·0	7·351	25·3	107·4	7·364	23·6	106·4	7·320
21·9	108·4	7·582	22·2	106·3	7·538	18·3	119·0	7·490
30·3	111·5	7·352	29·8	109·6	7·235	22·5	98·9	7·243
24·5	93·1	7·421	21·2	103·3	7·175	20·6	101·4	7·109
30·7	107·9	7·492	19·3	127·3	7·313	19	122·4	7·253
25·4	107·4	7·477	23·8	113·2	7·260	22·2	116·4	7·235
$26·4 \pm 1·4$	$105·1 \pm 2·7$	$7·446 \pm 0·036$	$23·6 \pm 1·5$	$111·2 \pm 3·5$	$7·314 \pm 0·052$	$21·0 \pm 0·9$**	$110·8 \pm 4·0$	$7·275 \pm 0·051$*

The total at the foot of each column represents Mean $\pm$ S.E.

* $P < 0·05$ ** $P < 0·01$

Table 5. The p_aCO_2, p_aO_2, and pH of arterial blood samples of 6 rabbits before, at the end and 30 minutes after the end of the haemorrhage.

TABLE 6

Normal			End of Bleed			30 min After Bleed		
p_aCO_2	p_aO_2	pH	p_aCO_2	p_aO_2	pH	p_aCO_2	p_aO_2	pH
60·0	58·4	7·175	60·3	59·2	7·219	61·2	74·8	7·227
57·5	84·2	7·266	56·1	90·8	7·272	53·2	98·9	7·281
57.8	62·4	7·257	55·7	62·1	7·253	53·8	81·6	7·250
55·8	85·2	7·279	45·3	83·8	7·273	34·9	96·9	7·310
50·1	86·6	7·182	45·8	90·4	7·211	33·7	97·4	7·229
58·2	78·9	7·361	49·2	73·9	7·298	59·1	80·1	7·360
56·6±1·4	75·9±5·1	7·253±0·028	52·1±2·5	76·7±5·7	7·254±0·014	49·3±4·9	88·3±4·3	7·276±0·021

The total at the foot of each column represents Mean ± S.E.

Table 6. The p_aCO_2, p_aO_2, and pH of arterial blood samples of 6 dogs before, at the end and 30 minutes after haemorrhage (the animals breathed 50% O_2 in air).

Effect of Haemorrhage on Blood pH, p_aCO_2 and p_aO_2.

a) Rabbits.

Comparison of pre-haemorrhage values of pH with values approximately half an hour after haemorrhage reveals a statistically significant ($P < 0{\cdot}05$) reduction from that of the control values. Only a slight reduction in pH values was seen at the end of bleed.

A slight decrease in p_aCO_2 was seen at the end of haemorrhage and this became significant ($P < 0{\cdot}01$) 30 minutes after the end of haemorrhage.

Slight increases in p_aO_2 were noted immediately after and 30 minutes after the end of haemorrhage (Table 5, Fig 3).

b) Dogs.

In six experiments the observations showed a slight increase in p_aO_2 together with slight decrease in p_aCO_2 neither of which were significant.

The pH showed little or no change even half an hour after the end of haemorrhage (Table 6 and Fig 3).

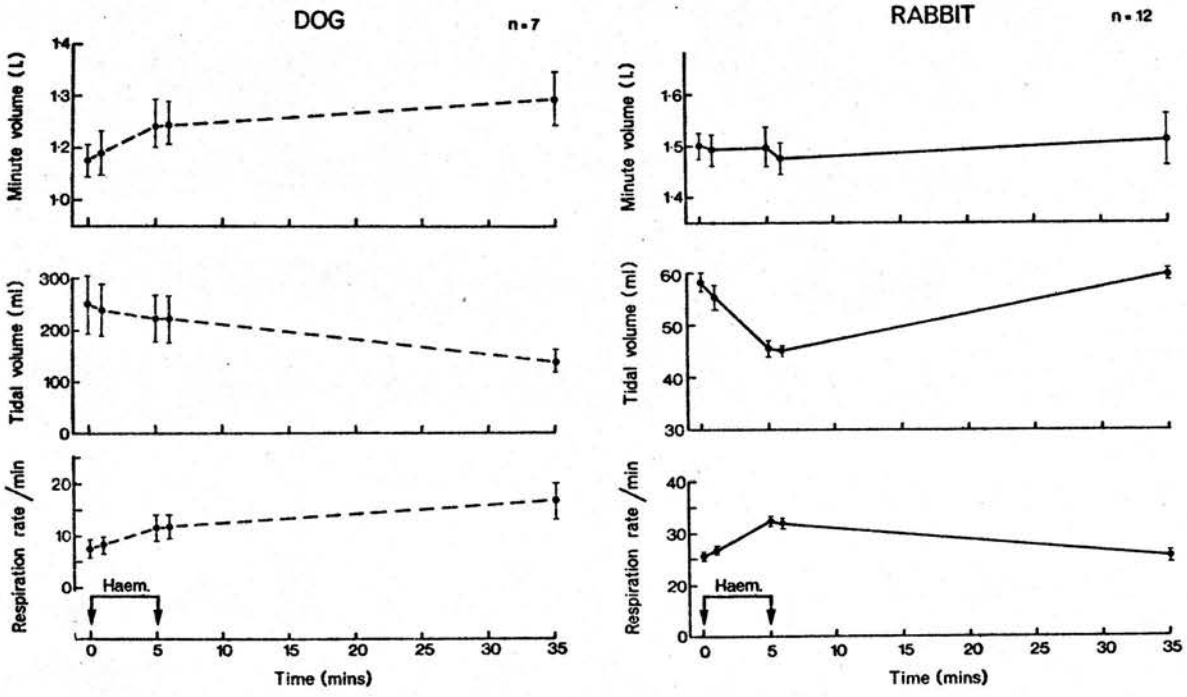
2. Effect of Haemorrhage on Respiration.

a) Rabbits.

In twelve experiments the tidal volume decreased significantly with an accompanying increase in respiratory rate during the course of haemorrhage. Respiratory minute volume was, with one or two exceptions, unchanged.

Figure 4.

The left panel shows the mean of the changes in the respiratory minute volume (l) in litres (upper), respiratory tidal volume (ml) in the middle, and the respiratory rate (lower) of 7 dogs.
The right panel shows the mean of the changes in the respiratory minute volume (l) in litres (upper), respiratory tidal volume (ml) in the middle, and the resipratory rate (lower) of 12 rabbits.
The arrows point to the time of start and finish of the haemorrhage (Haem).
Bars represent standard errors.


DOG
n = 7
RABBIT
n = 12
Minute volume (L)
Tidal volume (ml)
Respiration rate /min
Haem.
Time (mins)

The exceptions showed a slight increase at the end of bleed. Both tidal volume and minute volume had returned to normal after 30 minutes and were unchanged by the retransfusion of the shed blood (Fig 4).

b) Dogs.

In seven experiments there was a significant linear decrease in tidal volume accompanying a significant increase in minute volume. Respiratory rate increased significantly at the end of haemorrhage and continued at this level 30 minutes after the bleed. When the shed blood was returned back to the animal a further significant increase in respiratory rate, tidal volume and consequently the minute volume occurred. This returned gradually to resting values (Fig 4).

Adrenal Medullary Responses to Haemorrhage.

1. Effect of Haemorrhage on Catecholamine Release.

a) Rabbits - Arterial blood superfusion.

Initially, blood from the carotid artery was superfused over the rat stomach strip. However, even very large hypotensive changes produced no significant change of tone in the strip, in spite of clear cardiovascular indications of increased sympathetic activity.

It was thought that the relatively long time between secretion into the blood and that blood reaching the test organ might have been long enough for inactivation of the catecholamines.

Trace 1.

Rat stomach strip superfused with vena caval blood from a 3·5 kg rabbit anaesthetized with pentobarbitone sodium 40 mg/kg b.w.. The sensitivity of the strip was checked by 0·5 μg adrenaline (AD.) intravenously. Haemorrhage was commenced and the rat stomach strip showed a relaxation which was not continuous throughout the period following the bleed. It showed a big contraction during the period and returned to normal base line before the transfusion of the shed blood was started. Hexamethonium was infused as indicated by the markers (Hexa.) 10 mg/kg b.w., a few minutes after the infusion was finished haemorrhage was performed in the same manner and same amount, no relaxation of the muscle strip was observed.

Changes in heart rate in the middle panel.

Changes in the arterial blood pressure recorded from the carotid artery is seen on the lower panel.

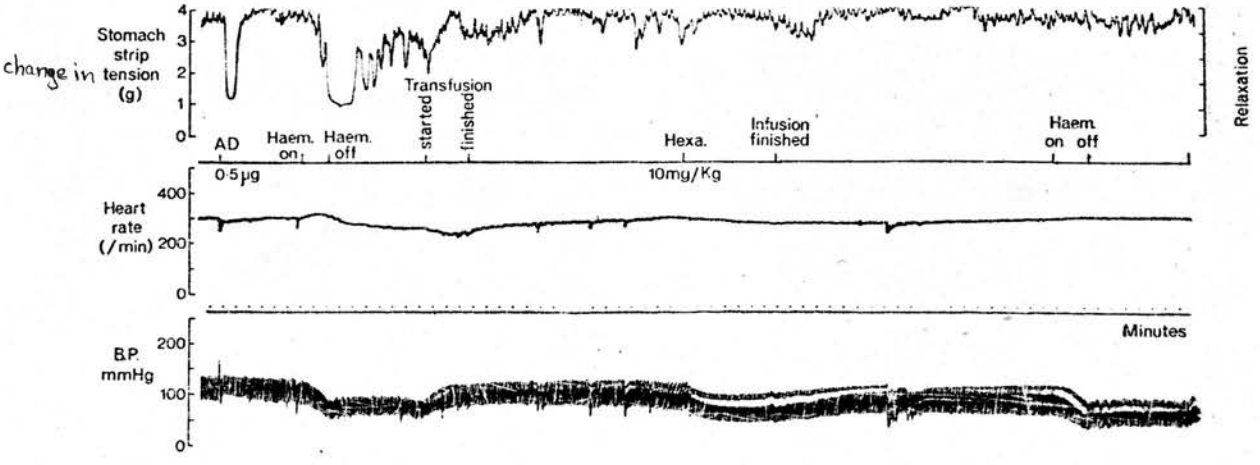

Stomach strip tension (g)
change in tension
4
3
2
1
0
AD
0·5µg
Haem. on
Haem. off
Transfusion started
finished
Hexa.
10mg/Kg
Infusion finished
Haem. on off
Relaxation
Heart rate (/min)
400
200
0
Minutes
B.P. mmHg
200
100
0

TABLE 7

Before bleed		Bleed				After bleed			
Control		1st Minute		End		1 Minute		20 Minutes	
B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %
107·5	10	92·5	40	70·0	76	77·5	74	77·5	30
102·5	20	82·5	30	60·0	90	62·5	80	75·0	40
112·5	20	102·5	20	57·5	100	52·5	100	67·5	20
105·0	24	92·5	28	62·5	86	67·5	80	82·5	30
87·5	20	75·0	20	60·0	80	60·0	80	65·0	50
87·5	30	77·5	30	52·5	70	55·0	70	70·0	30
100·0	28	72·5	36	62·5	60	65·0	46	70·0	30
85·0	22	80·0	22	57·5	50	60·0	100	75·0	40
92·5	40	80·0	40	65·0	90	67·5	80	77·5	30
110·0	25	97·5	25	77·5	74	85·0	60	105·0	40
97·5	20	90·0	20	55·0	25	57·5	25	65·0	25
112·5	28	102·5	28	55·0	70	60·0	55	75·0	40
$100·0 \pm 2·9$	$23·9 \pm 2.1$	$80·8 \pm 7·9^{*}$	$28·3 \pm 2·1$	$61·3 \pm 2·0^{**}$	$71·0 \pm 6·1^{**}$	$64·2 \pm 2·7^{**}$	$70·8 \pm 6·2^{**}$	$75·4 \pm 3·1^{**}$	$33·8 \pm 2·4$

Total at the foot of each column represents the Mean $\pm$ S.E.

* $P < 0·05$ ** $P < 0·001$

Table 7. Mean blood pressure (B.P.) and rat stomach strip relaxation (R.S.S.Relax %) before, 1st minute during, at the end, one minute after and 20 minutes after haemorrhage in 12 rabbits.

A new approach was devised as described in the methods in which blood from the inferior vena cava just central to the adrenal venous out-flow was collected and passed over the stomach strip preparation.

Twelve observations in 12 rabbits revealed a significant ($P < 0{\cdot}05$) decrease of blood pressure of $20{\cdot}8 \pm 8{\cdot}39$ (S.E. of difference) mmHg during the first minute of bleed. During this time no significant relaxation of the superfused rat stomach strip occurred, but as has already been noted, a quantitative increase in heart rate occurred. At the end of the bleed, which lasted approximately 5 minutes, the mean arterial blood pressure was further reduced $19{\cdot}58 \pm 8{\cdot}13$ (S.E. of difference) mmHg from that of the first minute of the bleed. At this stage a usually pronounced relaxation of the rat stomach strip had occurred. The change in tone was highly significant $47{\cdot}08 \pm 6{\cdot}41$ (S.E. of difference)% relaxation ($P < 0{\cdot}001$). This suggested a significant release of catecholamines from the adrenal medulla. It should be noted that there was a delay of about 60 seconds between the adrenal medullary release of catecholamine and its action on the stomach strip due to the "dead space" of the flow circuit.

At 15 - 20 minutes after the end of the bleed, blood pressure had recovered slightly, although still significantly below control levels. However, the rat stomach strip remained in relaxation during the first few minutes after the end of the bleed but then showed a gradual return to its resting tone after about 15 minutes

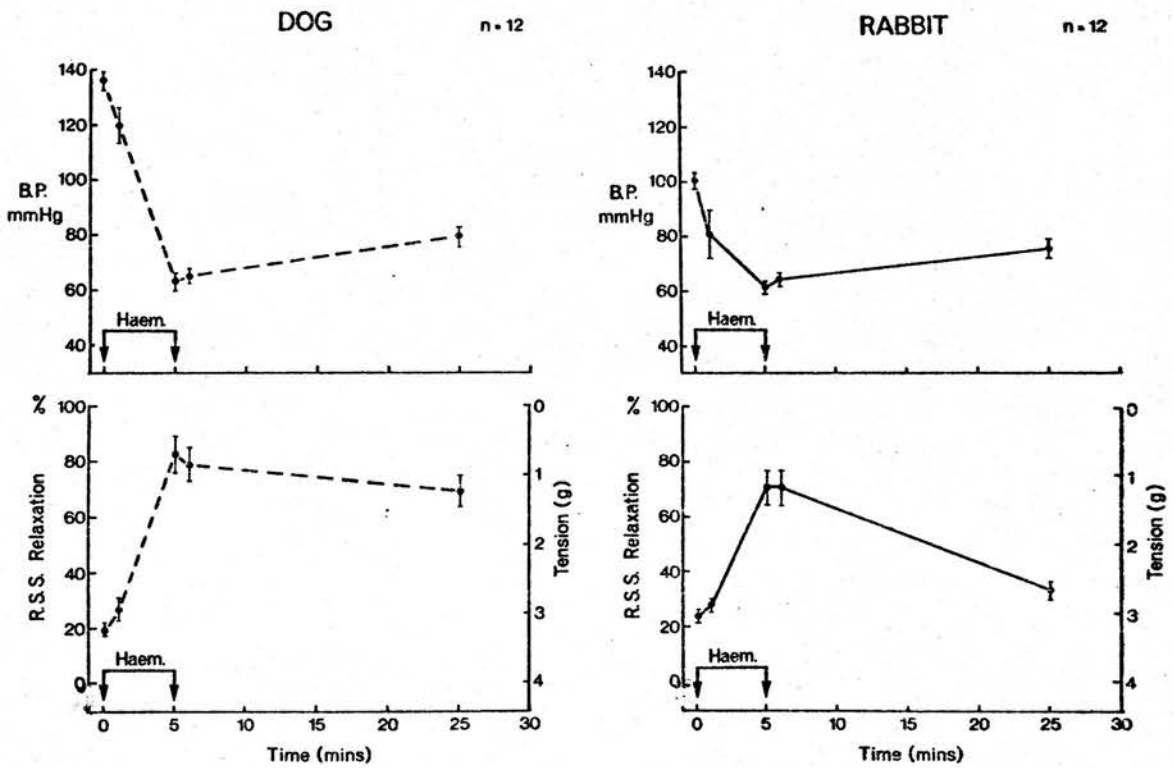
Figure 5.

The left panel (upper) represents the mean of arterial blood pressure (B.P.) mmHg of 12 dogs, left lower shows the mean of the changes in tension of a rat stomach strip (R.S.S. Relaxation %) superfused with vena caval blood from the same dogs, before, at one minute during, at the end, one minute, and 20 minutes after the end of haemorrhage.

The right panel (upper) represents the mean of the arterial blood pressure (B.P.) mmHg of 12 rabbits, the right panel (lower) shows the mean of the changes in tension of a rat stomach strip (R.S.S. Relaxation %) superfused with vena caval blood from the same rabbits, before, at one minute during, at the end, one minute after, and 20 minutes after haemorrhage.

The two arrows point to the start and finish of bleed.

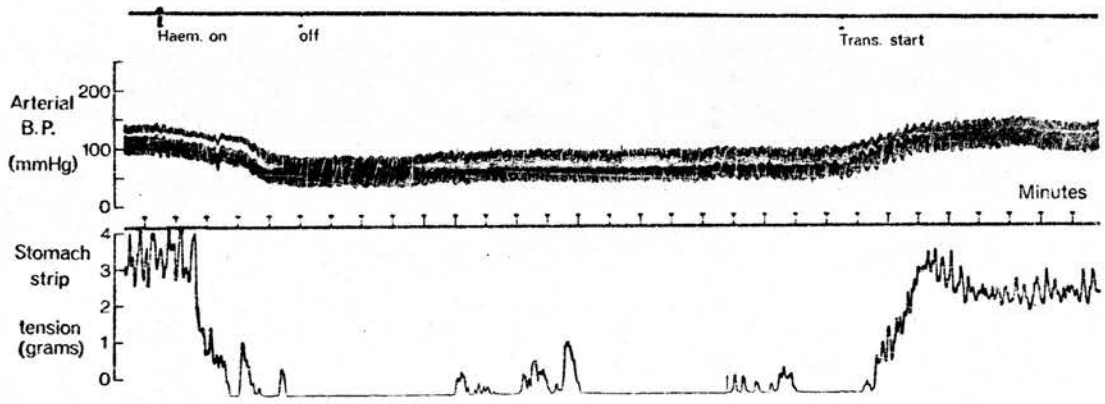
Bars represent standard errors.


DOG
n = 12
RABBIT
n = 12
B.P. mmHg
Haem.
% R.S.S. Relaxation
Tension (g)
Time (mins)

Trace 2.

Rat stomach strip in the lower panel superfused with vena caval blood from a 12·4 kg dog anaesthetized with pentobarbitone sodium. The relaxation of the rat stomach strip started a few minutes after haemorrhage and continued throughout the following period until the transfusion of the shed blood started, then the muscle strip returned almost to its control base line.

Arterial blood pressure recorded by 4 channel Devices recorder from the carotid artery is shown on the upper panel of the trace.


Haem. on
off
Trans. start
Arterial
B.P.
(mmHg)
200
100
0
Minutes
Stomach
strip
tension
(grams)
4
3
2
1
0

in most experiments. Observations of the condition of the superfused tissue during the 15 - 20 minites following the end of the haemorrhage showed a considerable level of spontaneous tonic activity. Some very large contractions reached or even exceeded the basal control levels of tone. Spontaneous activity settled down about 20 minutes after the end of the bleed and after the shed blood was returned back to the animal (Table 7, Figure 5 and Trace 1).

b) Dogs.

Similar experiments carried out in dogs showed some differences from rabbits. Twelve observations were made in twelve animals.

One minute after the onset of haemorrhage mean arterial blood pressure was lowered significantly $16{\cdot}0 \pm 7{\cdot}17$ (S.E. of difference) mmHg from the control ($P < 0{\cdot}05$) but there was no significant relaxation of the rat stomach strip. At the end of the haemorrhage which took between 5 - 8 minutes, arterial blood pressure was further reduced $56{\cdot}87 \pm 6{\cdot}67$ (S.E. of difference) mmHg accompanied by a significant relaxation of the superfused rat stomach strip of up to $63{\cdot}16 \pm 6{\cdot}9$ (S.E. of difference)% from the control value ($P < 0{\cdot}001$). The relaxation of the strip was maintained at more or less steady levels during the 15 - 20 minutes after the end of the bleed. With two exceptions, relaxation of the stomach strip remained significantly different from control levels

TABLE 8

Before Haemorrhage Control		During Haemorrhage 1st Minute		End		After Haemorrhage 1 Minute		30 Minutes	
B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %
135·0	40	65·0	60	60·0	96	62·5	96	72·5	70
125·0	20	112·5	30	80·0	96	80·0	84	95·0	84
115·0	20	97·5	30	72·5	100	75·0	100	87·5	80
140·0	30	125·0	40	52·5	100	52·5	80	60·0	80
150·0	14	150·0	10	60·0	54	60·0	65	90·0	54
157·5	12	140·0	20	55·0	100	57·5	100	75·0	40
137·5	24	125·0	28	60·0	88	60·0	80	75·0	80
150·0	14	130·0	20	62·5	30	75·0	30	85·0	30
135·0	10	130·0	10	72·5	74	75·0	74	90·0	74
127·5	20	125·0	30	65·0	92	65·0	92	75·0	90
135·0	14	120·0	30	60·0	66	60·0	60	75·0	60
125·0	16	120·0	16	57·5	96	60·0	96	72·5	90
$136·0 \pm 3·5$	$19·5 \pm 2·5$	$120·0 \pm 6·3^{*}$	$27·0 \pm 4·0$	$63·1 \pm 2·3^{**}$	$82·7 \pm 6·5^{**}$	$65·2 \pm 2·5^{**}$	$78·8 \pm 6·2^{**}$	$79·4 \pm 2·9^{**}$	$69·3 \pm 5·6^{**}$

Total at the foot of each column represents the Mean $\pm$ S.E.

* $P < 0·05$ ** $P < 0·001$

Table 8. Blood pressure (B.P.) and the rat stomach strip relaxation (R.S.S.Relax %) before, 1st minute during, at the end, one minute after and 30 minutes after haemorrhage in 12 dogs.

($P < 0{\cdot}001$), suggesting a release of catecholamine throughout the whole period of hypotension even though some restoration of blood pressure due to compensatory mechanisms had occurred following the end of the bleed and before the return of shed blood to the animal. The superfused tissue lost most of its spontaneous activity in most of our experiments during this period and appear as a straight line with a few small contractions on the trace (Trace 2).

On retransfusing the shed blood to the animal the tension of the superfused strip rapidly returned to the resting base-line level or a little higher. This return of tone appeared to occur when only a small quantity of blood had been returned and was accompanied by a return of spontaneous contractile activity. Blood pressure returned much more slowly to resting levels, usually not reaching normal levels until all the blood had been retransfused (Table 8, Fig 5).

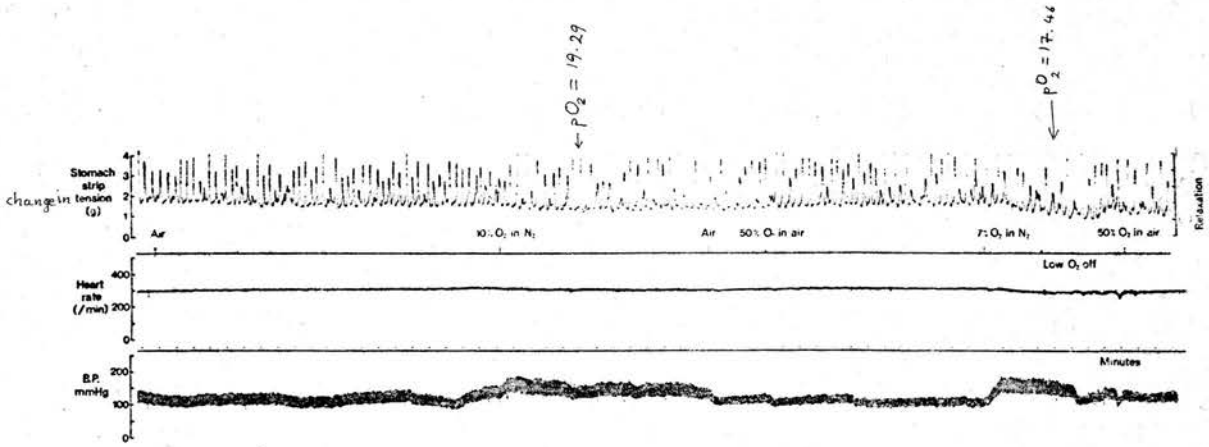
Reduction of pO_2.

One of the few factors apart from the catecholamines which is alleged to produce a reduction in tone of the stomach strip preparation is a fall in the pO_2 of the perfusate.

The venous blood in the rabbit in particular often appeared desaturated after haemorrhage procedures had been completed. Several experiments were carried out

Trace 3.

Rat stomach strip superfused with vena caval blood from a 2·6 kg rabbit anaesthetized with pentobarbitone sodium 40 mg/kg b.w. The rabbit was allowed to inspire normal air, 10% O_2 in N_2, 50% O_2 in air and 7% O_2 in N_2, no significant change in tension of the muscle strip was observed, the heart rate in the middle panel and the arterial blood pressure showed an increase during low oxygen inhalation.


← pO_2 = 19.29
pO_2 = 17.44 →
Stomach strip change in tension (g)
4
3
2
1
0
Relaxation
Air
10% O_2 in N_2
Air
50% O_2 in air
7% O_2 in N_2
50% O_2 in air
Low O_2 off
Heart rate (/min)
400
200
0
Minutes
B.P. mmHg
200
100
0

in which the inspired oxygen supply was restricted in order to produce severe desaturation of the venous blood in particular. Measurements of both arterial pO_2 and the pO_2 of the venous perfusion circuit were made. The effect on the tone of the stomach strip of a fall in pO_2 did not produce changes comparable to those seen after haemorrhage until a pO_2 of approximately 15 mmHg (Trace 3). In all our experiments venous pO_2 always exceeded 20 mmHg and was usually in the range 25 - 30 mmHg pO_2.

As a further precaution the rabbits breathed 50% oxygen/air mixture to maintain venous pO_2 at the highest possible level.

Ligation of Adrenal Veins.

a) Rabbit.

The circulatory isolation of the adrenal glands particularly that on the right side is not easy in this species. The right adrenal is almost invariably closely applied to the inferior vena cava with numerous small venous connections between the gland and the vein. Dissecting these small branches individually for ligation proved quite impossible, several rabbits dying as the vena cava ruptured. The vessels had to be occluded by applying a fine pair of artery forceps across the whole leash of small vessels. Isolation and occlusion of the venous outflow of the left adrenal gland was relatively easy and could be achieved by using bulldog clips (Diagram II).

In groups of three rabbits the adrenal veins were

Diagram 2.

Occlusion of the adrenal veins in the rabbit.

1 - The inferior vena cava.

2 - The right renal vein.

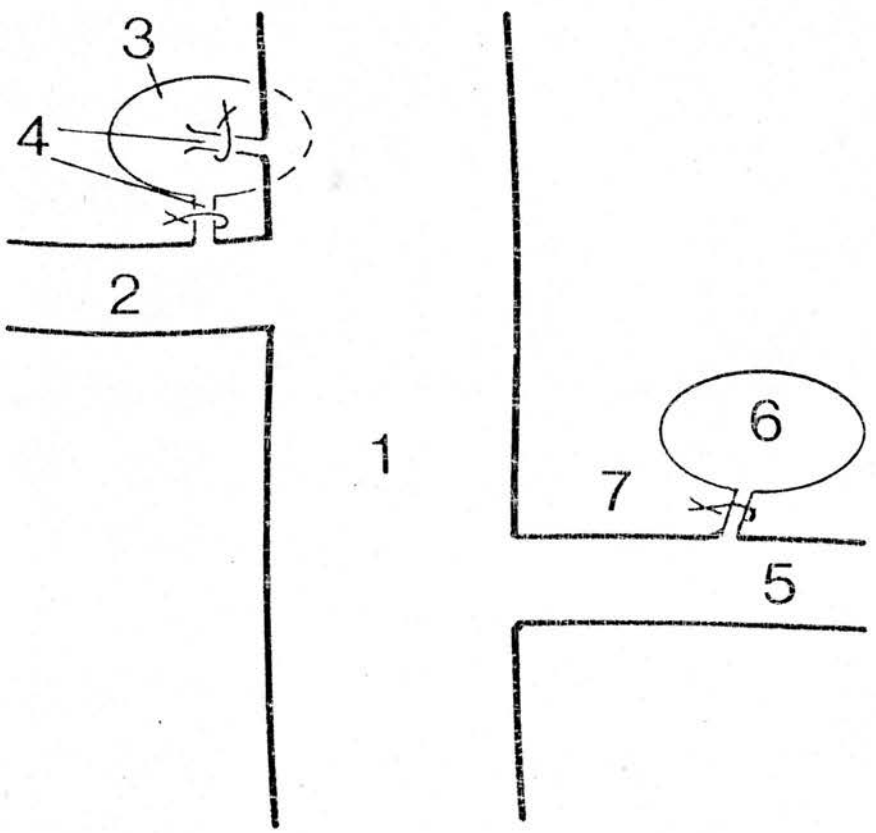
3 - The right adrenal gland.

4 - Two small branches of the left adrenal veins.

5 - Left renal vein.

6 - Left adrenal gland.

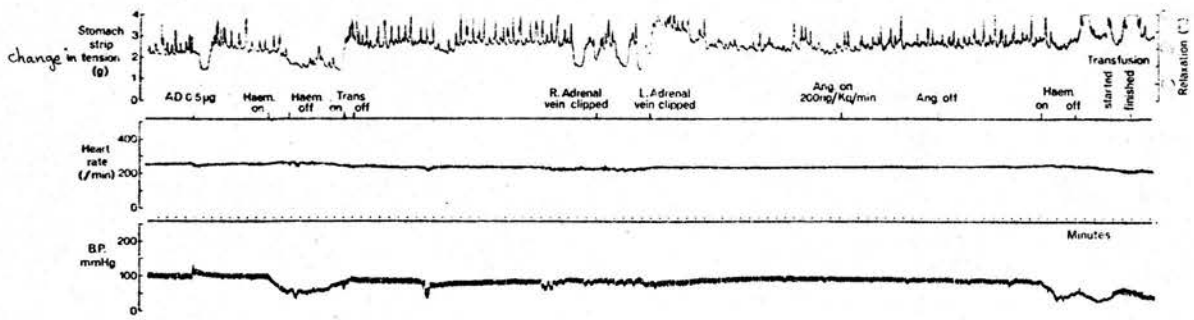
7 - Left adrenal vein.


3
4
2
1
6
7
5

Trace 4.

Rat stomach strip superfused with vena caval blood from a 2·5 kg rabbit anaesthetized with pentobarbitone sodium, the sensitivity of the strip was checked by test dose of adrenaline (AD.) 0·5 µg. i.v.. Normal haemorrhage showed relaxation of the stomach strip which then returned back to normal base line after blood transfusion. When the adrenal veins were clipped, pulling on the glands caused catecholamines release which caused transient relaxation of the strip. Bleed was carried out after the adrenal veins were clipped but it did not cause any change in the muscle strip tension.

The middle panel shows changes in heart rate during the experiment, lower panel shows changes of the arterial blood pressure which was recorded from the carotid artery by a Devices recorder.


Stomach strip
Change in tension (g)
4
3
2
1
0
A.D.C. 5µg
Haem. on
Haem off
Trans on
off
R. Adrenal vein clipped
L. Adrenal vein clipped
Ang on 200ng/Kg/min
Ang off
Haem on
off
Transfusion started
finished
Relaxation
Heart rate (/min)
400
200
0
Minutes
B.P. mmHg
200
100
0

dissected free and the venous outflow from the adrenal occluded as described above. During the occlusion procedure manipulation and pulling on the gland led to a small but significant relaxation of the rat stomach strip which suggested a significant release of catecholamine.

Following a standard haemorrhage the results revealed no significant change in tension of the stomach strip during or in the time following the bleed even though blood pressure remained significantly reduced (Trace 4).

The Response of Animals to Autonomic Ganglionic Blockade.

Evidence exists (Brown, 1967; Flacke and Gillis, 1968) for sympathetic ganglia that there are a population of muscarinic post ganglionic nerve cells as well as the classically accepted nicotinic receptors.

It was decided to see whether muscarinic blockade affected the release of catecholamine just shown. Because the (-) hyoscine methyl bromide used for muscarinic blockade has longer action than hexamethonium which has a maximum of about 45 minutes action, it was decided to use muscarinic blockade first.

Response of Animals with Muscarinic Blockade.

After administration of (-) hyoscine methyl bromide 5 mg/kg body weight, a dose capable of total muscarinic receptor blockade, the mean arterial blood pressure in some experiments fell slightly but returned to normal a few minutes after

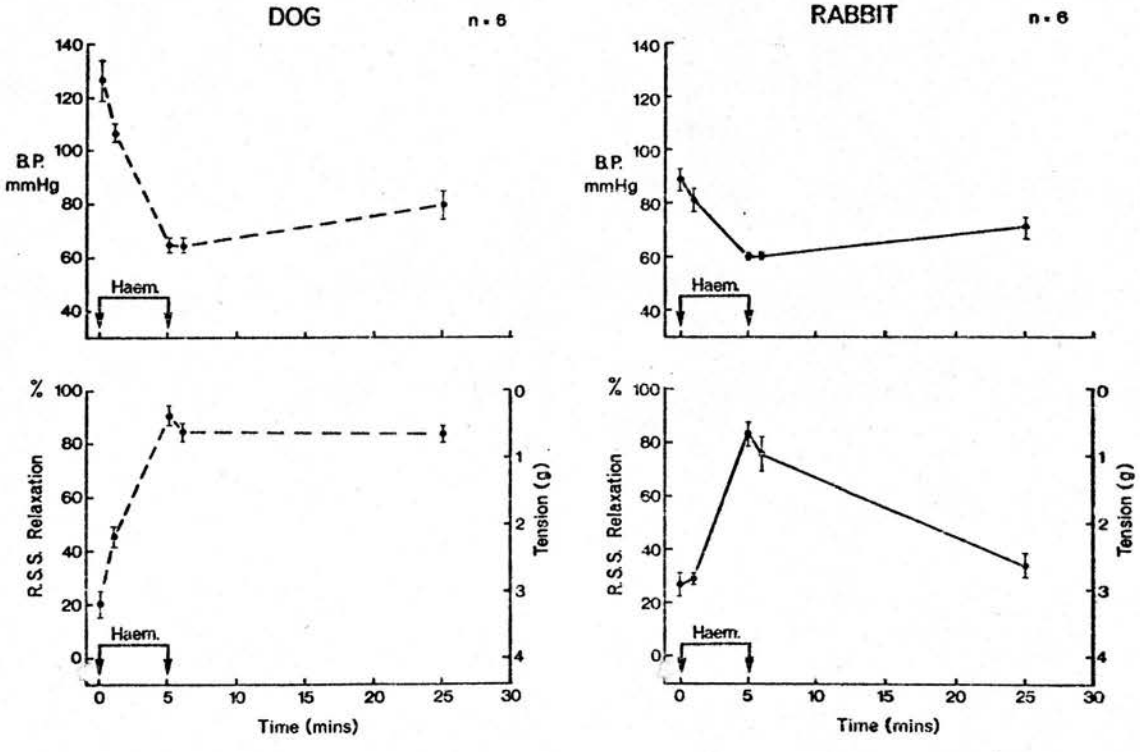
Figure 6.

The left panel (upper) represents the mean of arterial blood pressure (B.P.) mmHg of 6 dogs treated with (-) hyoscine methyl bromide 5 mg/kg, b.w. The left (lower) shows the mean changes in tension of rat stomach strips (R.S.S. Relaxation) superfused with vena caval blood from the same dogs, before, at one minute during, at the end of, one minute, and 20 minutes after the haemorrhage.

The right panel (upper) represents the mean of arterial blood pressure (B.P.) mmHg of 6 rabbits treated with hyoscine methyl bromide 5 mg/kg, b.w. The right (lower) shows the mean changes in tension of rat stomach strips (R.S.S. Relaxation) superfused with vena caval blood from the same rabbits, before, one minute during, at the end, one minute after, and 20 minutes after the haemorrhage.

The arrow points to the start and finish of bleed.

Bars represent standard errors.


DOG
n = 6
RABBIT
n = 6
B.P. mmHg
140
120
100
80
60
40
Haem.
% R.S.S. Relaxation
100
80
60
40
20
0
Tension (g)
0
1
2
3
4
0
5
10
15
20
25
30
Time (mins)

Trace 5.

Upper: Rat stomach strip superfused with vena caval blood from a 3·1 kg rabbit anaesthetized with pentobarbitone sodium 40 mg/kg. Hyoscine methyl bromide (Hyoscine) 5 mg/kg b.w. was infused i.v., after a few minutes resting period, bleeding was commenced and the blood pressure lowered to 50 mmHg, the muscle strip showed a relaxation at the end of the bleed. When the shed blood returned back to the animal the stomach strip returned to its previous baseline.
Lower: A continuation of the upper trace showing adrenaline (AD.) 0·5 μg test dose which was injected i.v. to check the sensitivity of the strip then carbachol 1 mg was injected i.v., a significant decrease in tension of the rat stomach strip was seen. Hexamethonium bromide (Hexa.) 10 mg/kg b.w. was superfused i.v. and another dose of carbachol 1 mg was injected i.v. but no relaxation of the strip was observed.

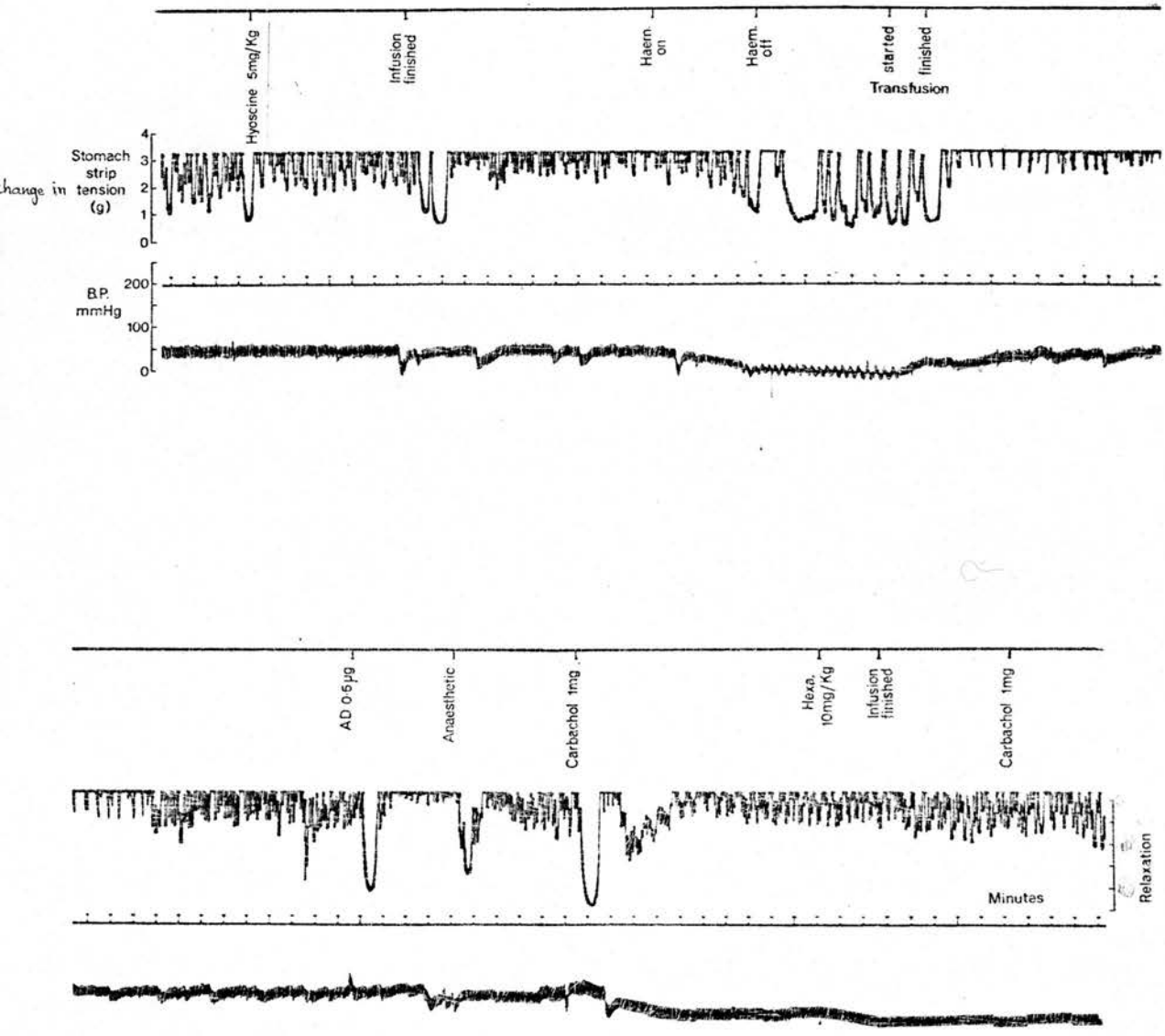

Hyoscine 5mg/Kg
Infusion finished
Haem. on
Haem. off
started
finished
Transfusion
Change in Stomach strip tension (g)
4
3
2
1
0
B.P. mmHg
200
100
0
AD 0.5µg
Anaesthetic
Carbachol 1mg
Hexa. 10mg/Kg
Infusion finished
Carbachol 1mg
Minutes
Relaxation

TABLE 9

Before bleed		Bleed				After bleed			
Control		1st Minute		End		1 Minute		20 Minutes	
B.P. mmHg	R.S.S Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %
80·0	30	75·0	30	55·0	68	55·0	68	87·5	44
107·5	34	100·0	36	60·0	86	60·0	78	77·5	38
92·5	20	87·5	30	60·0	100	60·0	100	65·0	50
87·5	22	80·0	22	62·5	90	62·5	82	70·0	22
82·5	28	75·0	28	60·0	86	62·5	78	65·0	30
85·0	28	72·5	30	62·5	72	62·5	50	62·5	24
89·2±4·1	27·0±2·1	81·7±4·3	29·3 ±1·8	60·0±1·1**	83·7±4·8**	60·4±1·2**	76·0±6·7**	71·3±3·9*	34·7±4·6

Total at the foot of each column represents the Mean ± S.E.

* $P < 0.01$ ** $P < 0.001$

Table 9. Mean arterial blood pressure (B.P.) and Rat stomach strip solution (R.S.S.Relax.%) before, 1st minute during, at the end, one minute after and 20 minutes after haemorrhage in 6 rabbits treated with Hyoscin emethyl bromide 5 mg/kg b.w.

the infusion stopped. In most of the experiments the infusion of hyoscine was accompanied by small but significant decrease of tone at the stomach strip which lasted a few minutes and returned to normal. Haemorrhage was then performed as described previously to find the effect of the muscarinic block.

a) Rabbits.

Six observations in separate experiments revealed slight but not significant reduction of mean arterial blood pressure with no change in tension of the rat stomach strip during the first minute of haemorrhage. At the end of the bleed (5 minutes after the start) the blood pressure was reduced to about 50 - 60 mmHg. The stomach strip showed significant ($P < 0{\cdot}001$) increase in relaxation of $56{\cdot}66 \pm 5{\cdot}27$ (S.E. of difference)% from that of the control value. One minute after the end of the bleed the mean arterial blood pressure remained unchanged but there was a slight decrease $7{\cdot}66 \pm 8{\cdot}28$ (S.E. of difference)% in the relaxation of the strip.

The blood pressure showed slight linear increase during the 15 - 20 minute period following the bleed ($11{\cdot}25 \pm 4{\cdot}06$ (S.E. of difference) mmHg) but still significantly below normal. The tone of the stomach strip gradually increased after the first minute and was back to normal base line within a few minutes, usually long before the retransfusion of the shed blood started (Table 9, Fig 6 and Trace 5).

Trace 6.

Arterial blood pressure was recorded from the carotid artery of a 12·5 kg dog anaesthetized with pentobarbitone sodium (lower panel). The upper panel shows a rat stomach strip superfused with vena caval blood from the same dog. Adrenaline (AD.) test dose was given i.v., the strip showed a relaxation and then hyoscine methyl bromide was infused in a dose of 5 mg/kg b.w., i.v.. After the blockade of muscarinic receptor with hyoscine, angiotensin (Ang.) infusion was carried out into the aorta in a dose of 200 ng/kg/min., relaxation of the strip was seen after two minutes of the infusion and the base line returned to normal after about two more minutes, the angiotensin infusion was stopped after 10 minutes. Haemorrhage was then carried out and relaxation of the strip happened which continued until the shed blood was retransfused to the animal.

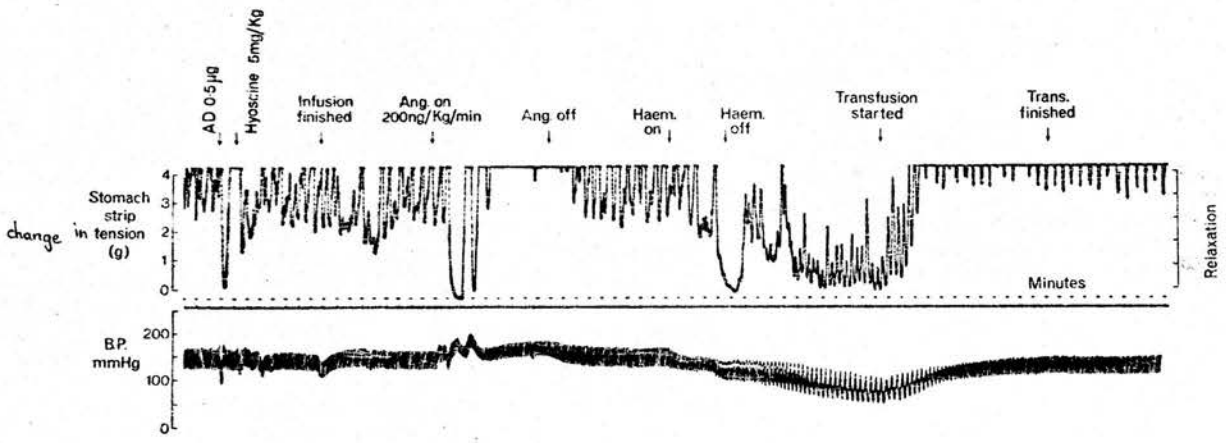

AD 0·5 µg
Hyoscine 5mg/Kg
Infusion finished
Ang. on 200ng/Kg/min
Ang. off
Haem. on
Haem. off
Transfusion started
Trans. finished
Stomach strip in tension (g)
change
4
3
2
1
0
Minutes
Relaxation
B.P. mmHg
200
100
0

TABLE 10

Before Haemorrhage		During Haemorrhage				After Haemorrhage			
Control		1st Minute		End		1 Minute		15 Minutes	
B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %
142·5	20	107·5	50	57·5	100	57·5	100	65·0	100
135·0	30	112·5	40	60·0	96	62·5	90	75·0	90
117·5	40	97·5	80	77·5	90	77·5	80	102·5	80
147·5	10	120·0	40	65·0	96	65·0	80	87·5	80
115·5	10	105·0	42	65·0	78	67·5	78	75·0	70
100·0	12	95·0	20	65·0	82	60·0	80	75·0	80
$126 \cdot 3 \pm 7 \cdot 5$	$20 \cdot 3 \pm 5 \cdot 0$	$106 \cdot 3 \pm 3 \cdot 8^{*}$	$45 \cdot 3 \pm 8 \cdot 0^{*}$	$65 \cdot 0 \pm 2 \cdot 8^{**}$	$90 \cdot 3 \pm 3 \cdot 6^{**}$	$65 \cdot 0 \pm 2 \cdot 9^{**}$	$84 \cdot 7 \pm 3 \cdot 5^{**}$	$80 \cdot 0 \pm 5 \cdot 4^{**}$	$83 \cdot 3 \pm 4 \cdot 2^{**}$

Total at the foot of each column represents the Mean $\pm$ S.E.

* $P < 0 \cdot 05$ ** $P < 0 \cdot 001$

Table 10. Blood pressure (B.P.) and rat stomach strip relaxation (R.S.S.Relax %) before, 1st minute during, at the end, one minute after and 15 minutes after haemorrhage in 6 dogs treated with (-) Hyoscin methyl bromide.

b) Dogs

The arterial blood pressure with one exception slightly lowered during the infusion of (-) hyoscin methyl bromide. The exception showed a marked drop in blood pressure during the infusion of the blockade. The stomach strip showed a significant reduction in tone during the infusion which remains for a few minutes before returning to normal.

In six dog experiments the results showed significant decrease of 20·08 $\pm$ 8·38 (S.E. of difference) mmHg in mean arterial blood pressure ($P < 0{\cdot}05$) during the first minute of haemorrhage together with a significant increase of 25 $\pm$ 9·48 (S.E. of difference)% in the relaxation of the rat stomach strip ($P < 0{\cdot}05$). This relaxation increased a further 45 $\pm$ 8·77 (S.E. of difference)%. Blood pressure decreased a further 41·25 $\pm$ 4·75 (S.E. of difference) mmHg at the end of bleed. During the following 15 - 20 minutes after the end of the bleed, blood pressure increased 15 $\pm$ 6·05 (S.E. of difference) mmHg from its value at the end of the bleed but was still significantly below control level.

When the shed blood was retransfused into the animal, blood pressure gradually returned to control level as the transfusion was completed. The relaxation of the rat stomach strip itself was still significantly ($P < 0{\cdot}001$) above normal together with a decrease in spontaneous activity during the whole period following the haemorrhage. When the shed blood was returned to the animal tone

quickly returned to control levels or slightly higher with a return of spontaneous contractility. This occurred after only a small amount of the shed blood had entered the circulation of the animal (Table 10, Figure 6, Trace 6).

The Effect of Haemorrhage after Nicotinic Blockade.

a) Rabbits.

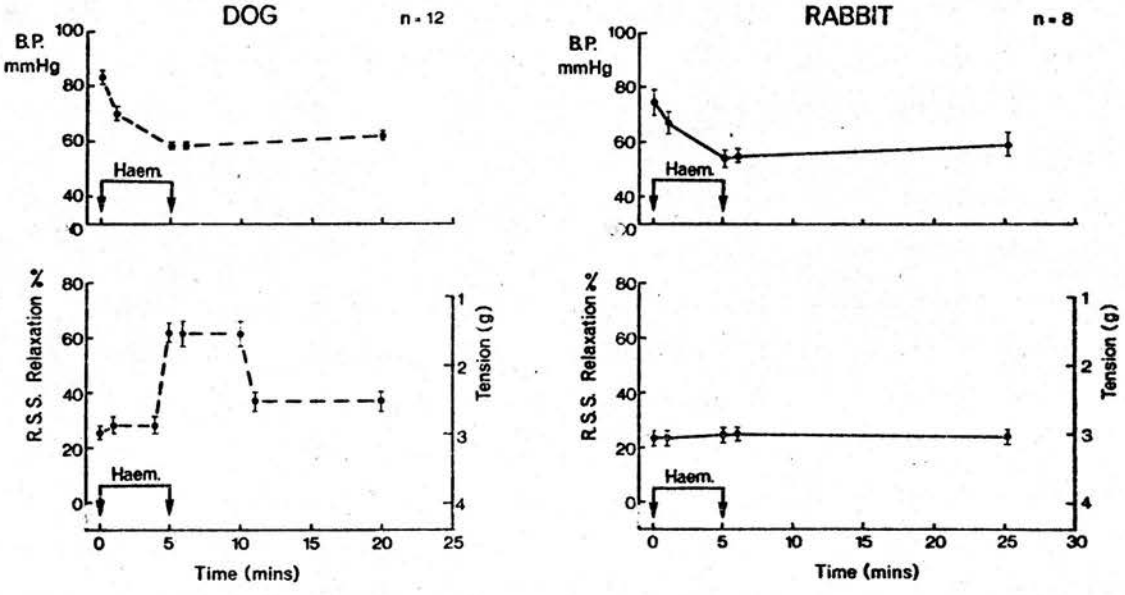
In a group of 8 animals hexamethonium bromide was administered intravenously in a dose of 10 mg/kg body weight, prepared in 20 ml saline and infused very slowly using an infusion pump. To be sure that the blockade was complete an extra small dose of hexamethonium was injected after the main dose. If the blood pressure did not fall further this was taken as an indication of total blockade.

In four rabbits of the group the arterial blood pressure was lowered significantly after the administration of the blocking agent. A dextran-40 drip was given to these animals to stabilize the blood pressure at an acceptable level before commencing the haemorrhage. In a further two rabbits the basal tone of the stomach strip became irregular and spontaneous contractile activity increased. In the other two animals relaxation of the perfused tissue occurred during the hexamethonium infusion. This reduction of tone was reversed when blood pressure stabilized using dextran.

When haemorrhage was carried out a slight but

Figure 7.

Left panel (upper) shows the mean of the arterial blood pressure (B.P.) mmHg of 12 dogs treated with hexamethonium bromide 10 mg/kg b.w. The left (lower) represents the mean of the changes in tension of a rat stomach strip (R.S.S. Relaxation %) superfused with vena caval blood from the same dogs, before, one minute during, at the end, one minute after, and 15 minutes after haemorrhage.
Right panel (upper) shows the mean of the arterial blood pressure (B.P.) mmHg of 8 rabbits treated with hexamethonium bromide 10 mg/kg b.w. The right (lower) shows the mean of the changes of tension of rat stomach strips (R.S.S. Relaxation %) superfused with vena caval blood from the same rabbits, before, one minute during, at the end, one minute after, and 20 minutes after haemorrhage.
Bars represent standard errors.


DOG
n = 12
B.P. mmHg
100
80
60
40
0
Haem.
R.S.S. Relaxation %
80
60
40
20
0
Haem.
1
2
3
4
Tension (g)
0
5
10
15
20
25
Time (mins)
RABBIT
n = 8
B.P. mmHg
100
80
60
40
0
Haem.
R.S.S. Relaxation %
80
60
40
20
0
Haem.
1
2
3
4
Tension (g)
0
5
10
15
20
25
30
Time (mins)

Trace 7.

The upper panel shows a rat stomach strip superfused with vena caval blood from a 3·5 kg rabbit anaesthetized with pentobarbitone sodium. Hexamethonium bromide (Hexa.) 10 mg/kg b.w. was infused intravenously to achieve blockade of nicotinic receptor. After the infusion finished, adrenaline (AD.) 0·5 μg test dose was injected i.v., the strip relaxed and returned back to normal base line quickly. When haemorrhage was carried out no relaxation of the stomach strip was observed.

The middle panel shows the changes in heart rate recorded by a Devices ratometer.

The lower panel shows arterial blood pressure recorded from the carotid artery using a pressure transducer.

change in

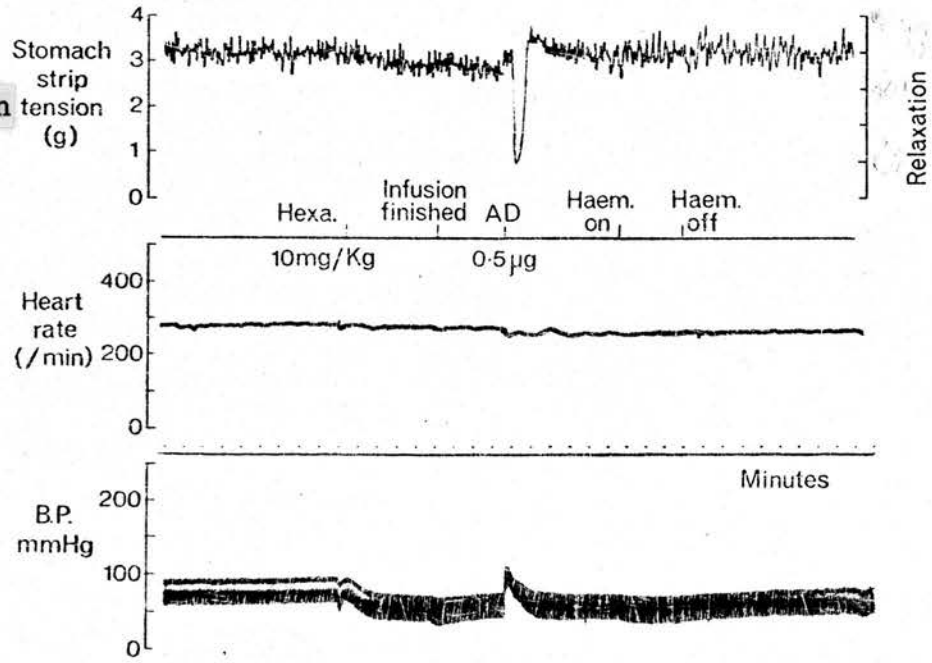

Stomach strip tension (g)
4
3
2
1
0
Relaxation
Hexa.
Infusion finished
AD
Haem. on
Haem. off
10mg/Kg
0·5µg
Heart rate (/min)
400
200
0
Minutes
B.P. mmHg
200
100
0

TABLE 11

Before Haemorrhage		Haemorrhage				After Haemorrhage			
Control		1st Minute		End		1 Minute		15 Minutes	
B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %
92.5	10	77·5	12	62·5	12	62·5	12	70·0	12
62·5	20	55·0	20	55·0	22	57·5	22	57·5	22
60·0	32	60·0	32	50·0	32	50·0	30	55·0	30
62·5	24	55·0	24	40·0	28	42·5	28	52·5	26
92·5	20	85·0	20	52·5	20	52·5	22	67·5	20
70·0	20	62·5	20	45·0	18	50·0	22	50·0	20
72·5	34	65·0	34	52·5	36	52·5	36	52·5	36
80·0	30	75·0	28	60·0	28	60·0	28	55·0	28
$74·1 \pm 4·6$	$23·8 \pm 2·8$	$66·9 \pm 3·9$	$23·8 \pm 2·6$	$53·4 \pm 2·9$ *	$24·5 \pm 2·8$	$54·7 \pm 2·5$ *	$25·0 \pm 2·5$	$58·8 \pm 2·9$ **	$24·3 \pm 2·6$

Total at the foot of each column represents the Mean $\pm$ S.E.

*$P < 0·01$. **$P < 0·02$.

Table 11. Blood pressure (B.P.) and rat stomach strip relaxation (R.S.S.Relax %) before, 1st minute during, at the end, one minute after and 15 minutes after haemorrhage in 8 rabbits treated with Hexamethonium bromide 10 mg/kg b.w.

insignificant reduction in blood pressure occurred during the first minute of the bleed. During this time there was no change in the tension of the superfused tissue. Starting from lower control values, the fall in blood pressure was much less than before blockade to reach a mean pressure of 50 - 60 mmHg. Nevertheless, the reduction in blood pressure of 13·43 $\pm$ 4·87 (S.E. of difference) mmHg was a statistically significant one ($P < 0{\cdot}01$), at the end of the bleed. Throughout the whole period of the bleed there was no significant increase in relaxation of the superfused rat stomach strip, suggesting no significant release of catecholamines from the adrenal medulla. One minute after the end of the bleed the mean arterial pressure showed no significant change and there was no significant change of tone of the superfused tissue. During the following 15 - 20 minutes after the bleed, blood pressure increased slightly due to internal compensation 4·07 $\pm$ 3·82 (S.E. of difference) mmHg from its value at the end of the bleed but was still significantly ($P < 0{\cdot}02$) below control levels. The rat stomach strip showed no change in tension during this period (Table 11, Figure 7 and Trace 7).

b) Dogs.

In a group of twelve animals the blood pressure was reduced significantly during the first minute of haemorrhage with no significant change in relaxation of the superfused tissue. At the end of the bleed with the

Trace 8.

A rat stomach strip superfused with vena caval blood from a 13·2 kg dog anaesthetized with pentobarbitone sodium. Hexamethonium bromide (Hexa.) 10 mg/kg b.w. was infused to block the nicotinic receptor, after the infusion was finished adrenaline (AD.) 0·5 μg test dose was injected i.v. to check the sensitivity of the strip. Angiotensin (Ang.) 400 ng/kg/min was infused into the aorta, after about two minutes of the infusion the muscle strip showed relaxation which did not last throughout the whole period of infusion and returned to control base line after about three minutes. The ang. infusion was stopped after ten minutes and haemorrhage was performed, again the stomach strip showed a transient relaxation continued only a few minutes after the bleed stopped and returned to control base line a long time before the shed blood was retransfused. After the whole blood was retransfused to the animal, another test dose of adrenaline (AD.) was injected i.v., and after a few minutes resting time a dose of 1 mg carbachol was injected i.v. after hyoscine infusion, the stomach strip showed relaxation, when the tension returned to control base line hexamethonium was infused and the same dose of carbachol (1 mg) was injected i.v., no relaxation was then noticed.
The lower panel shows the changes in the arterial blood pressure which was recorded from the carotid artery during the experiment.

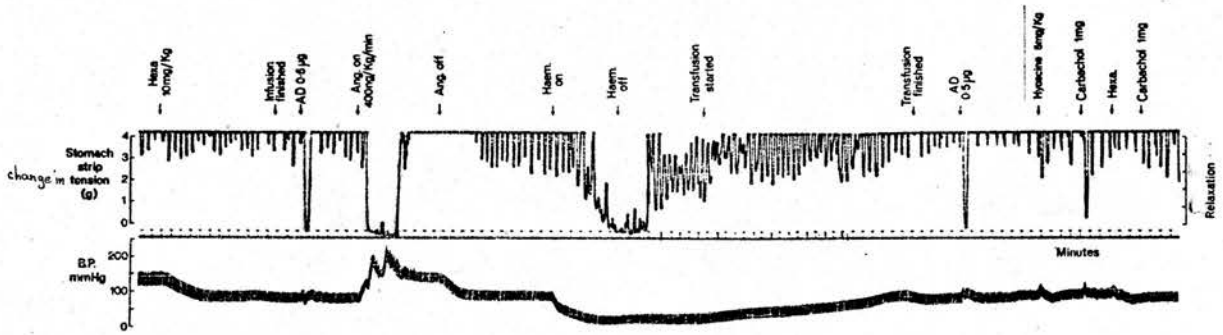

Hexa 10mg/Kg
Infusion finished
AD 0.5 µg
Ang. on 400ng/Kg/min
Ang. off
Haem. on
Haem. off
Transfusion started
Transfusion finished
AD 0.5µg
Hyoscine 4mg/Kg
Carbachol 1mg
Hexa.
Carbachol 1mg
Stomach strip change in tension (g)
4
3
2
1
0
Relaxation
Minutes
B.P. mmHg
200
100
0

TABLE 12

Before Haemorrhage		During Haemorrhage				After Haemorrhage			
Control		1st Minute		End		1 Minute		15 Minutes	
B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %
92·5	16	85·0	28	60·0	80	60·0	80	72·5	18
60·0	40	60·0	40	55·0	70	55·0	70	60·0	42
87·5	20	65·0	30	55·0	80	55·0	80	50·0	60
80·0	30	65·0	30	50·0	40	50·0	40	60·0	36
82·5	40	67·5	48	60·0	72	62·5	70	65·0	68
85·0	30	75·0	42	62·5	70	62·5	70	65·0	68
90·0	30	65·0	30	60·0	30	60·0	30	65·0	20
92·5	14	87·0	24	60·0	68	60·0	68	70·0	62
80·5	22	75·0	20	60·0	62	60·0	62	62·0	18
81·0	20	70·0	22	58·0	58	58·0	56	60·0	26
83·0	20	67·0	16	58·0	60	58·0	62	59·0	14
85·5	22	62·5	12	62·0	52	64·0	52	61·5	16
$83 \cdot 3 \pm 2 \cdot 5$	$25 \cdot 3 \pm 2 \cdot 5$	$70 \cdot 3 \pm 2 \cdot 5^{*}$	$28 \cdot 5 \pm 3 \cdot 1$	$58 \cdot 4 \pm 1 \cdot 0^{**}$	$61 \cdot 8 \pm 3 \cdot 5^{**}$	$58 \cdot 8 \pm 1 \cdot 1^{**}$	$61 \cdot 7 \pm 4 \cdot 4^{**}$	$62 \cdot 5 \pm 1 \cdot 7^{**}$	$37 \cdot 3 \pm 3 \cdot 7^{**}$

Total at the foot of each column represents the Mean $\pm$ S.E.

$^{*}P < 0{\cdot}05$ $^{**}P < 0{\cdot}001$

Table 12. Blood pressure (B.P.) and rat stomach strip relaxation (R.S.S.Relax %) before, 1st minute during, at the end, one minute after and 15 minutes after haemorrhage in 12 dogs.

blood pressure reduced to approximately 50 - 60 mmHg there was a significant increase in stomach strip relaxation of 36·5 $\pm$ 5·043 (S.E. of difference)% from control level ($P < 0{\cdot}001$) which appeared about one minute before the end of bleed. No further significant blood pressure or strip tension changes were noted at one minute after the end of the bleed. In the following 10 - 15 minutes following the completion of the bleed, arterial blood pressure recovered slightly but remained significantly below the control level ($P < 0{\cdot}001$). The relaxation of the stomach strip remained significantly above normal ($P < 0{\cdot}001$) in five dogs only during the 15 - 20 minutes following the end of the bleed while in the other seven the tone of the strip returned to normal or slightly below normal after 5 - 8 minutes of the end of the bleed and remained in position throughout the whole rest period suggesting that the release of catecholamines was a transient one and not continuous all over the period.

When the shed blood was retransfused into the animals the blood pressure as well as the tension of the strip returned to control level.

As a matter of fact, the relaxation of stomach strip during its maximum at the end of the bleed was much less than that before blockade or with muscarinic blockade suggesting that the release of catecholamine was reduced after hexamethonium (Table 12, Figure 7 and Trace 8).

Ligation of renal veins following nicotinic blockade was carried out as described previously in three dogs.

The response of the stomach strip preparation to the standard haemorrhage procedure was then noted. No significant change of muscle tone occurred (Trace 8).

Effect of Carbachol Injection on Catecholamine Release.

A single small dose (1 mg) of carbachol was injected in groups of two rabbits and two dogs after muscarinic blockade. There was a significant relaxation ($P < 0{\cdot}001$) of the stomach strip (Traces 5 and 8).

The same dose was repeated after administration of hexamethonium to achieve nicotinic receptor blockade. Following blockade there was no significant relaxation in the superfused circuit (Traces 5 and 8).

Effect of Haemorrhage on Angiotensin Levels.

The above results in the dogs appeared to indicate that catecholamine release from the adrenal medulla during haemorrhage was not entirely under the control of the sympathetic nerves.

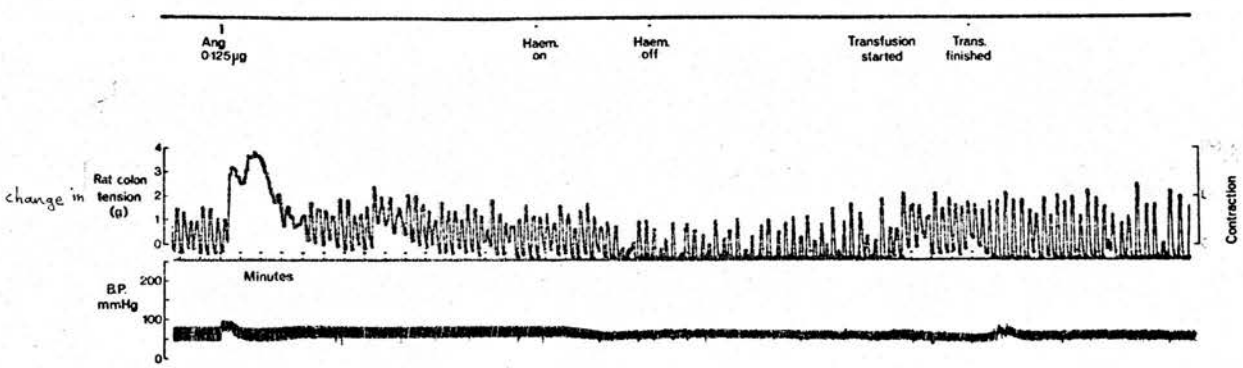
The possibility was that other humoral substances released during the haemorrhagic stimulus in turn caused release of catecholamines. It is well known (see previous literature) that angiotensin is released during haemorrhage in dogs and that it is capable of releasing adrenal medullary catecholamines.

It was decided therefore, to study the release of angiotensin in both species during haemorrhage and its possible role in release of catecholamine.

Trace 9.

A rat colon superfused with arterial blood from the carotid artery of a 2·6 kg rabbit anaesthetized with pentobarbitone sodium. A check dose of angiotensin (Ang.) 0·125 μg was injected intravenously and a contraction of the colon was noticed. After the colon returned to control base line a haemorrhage was carried out but no contraction was noticed either during the bleed or after. The shed blood was then retransfused to the animal.

The lower panel shows the arterial blood pressure which was recorded from the carotid artery.


1
Ang
0·125µg
Haem.
on
Haem.
off
Transfusion
started
Trans.
finished
change in
Rat colon
tension
(g)
4
3
2
1
0
Contraction
Minutes
B.P.
mmHg
200
100
0

a) Rabbits.

In a group of four rabbits the rat ascending colon strip (Regoli and Vane, 1964) was used to investigate the changes in blood angiotensin level during haemorrhage. Blood for superfusing the strip was drawn from the vena cava above the adrenal venous outflow, in the same manner as for the stomach strip. The rat colon is highly sensitive to angiotensin II which causes it to contract.

No significant change in the tension of the colon strip was revealed during haemorrhage or during the 30 minutes following the bleed, even though the blood pressure reduced significantly at the end of the bleed (Trace 9).

It was thought that angiotensin generation in the circulation might require a longer time and that angiotensin might be found in the arterial blood more readily than in venous blood. In two rabbits blood from the carotid artery was drawn for superfused rat colon, but no significant change in the tension of the colon strip was observed during haemorrhage or in the 30 minutes after the bleed was completed.

b) Dogs.

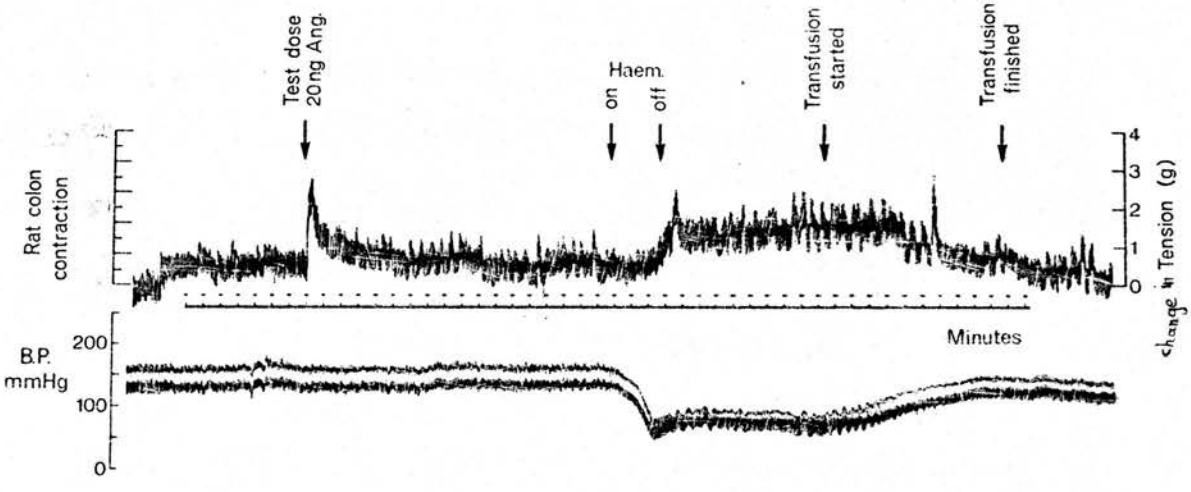
In a group of three dogs blood also taken from the carotid artery was superfused over the colon strip preparation.

At the end of a moderate haemorrhage when blood pressure was lowered to about 60 mmHg a marked increase

Trace 10.

A rat colon superfused with arterial blood from the carotid artery of a 13·5 kg dog anaesthetized with pentobarbitone sodium. A test dose of 20 ng angiotensin (Ang.) was injected in the inflow circuit, the rat colon contracted and returned back to control base line. A few minutes later, haemorrhage was carried out, the colon contracted and continued in its contraction until the blood was retransfused to the animal then the colon returned to its control base line.

The lower panel shows the changes in arterial blood pressure which was recorded from the femoral artery.


Test dose
20ng Ang.
Haem.
on
off
Transfusion
started
Transfusion
finished
Rat colon
contraction
4
3
2
1
0
change in Tension (g)
Minutes
B.P.
mmHg
200
100
0

in the tension of the strip was observed. This contraction continued until the blood was returned to the animal when the contraction disappeared and tension of the muscle strip returned to control levels (Trace 10).

In one of these experiments laparotomy was performed, both renal veins dissected free and loose ties passed around them allowing their ligation at a later stage. Before commencing the bleed both renal veins were ligated and haemorrhage then carried out until the blood pressure was lowered to about 60 mmHg. No increase in the tension of the rat colon was observed when the ties were released. A contraction of the strip was noticed about one minute after the ties had been released.

These experiments seem to indicate no significant release of angiotensin in the rabbit and a marked release in the dog.

It was still possible that angiotensin releases catecholamine. The responses of the stomach strip preparation were followed in rabbits and dogs during progressively increasing angiotensin infusion.

Effects of Angiotensin Infusion on Catecholamine Release.

a) Rabbits.

In a group of ten rabbits the results of angiotensin infusion have been studied. Infusions were carried out by intra-arterial infusion into the aorta just central to the arterial inflow to the adrenal glands. Infusions started with small doses 100 ng/kg/min and increased

Figure 8.

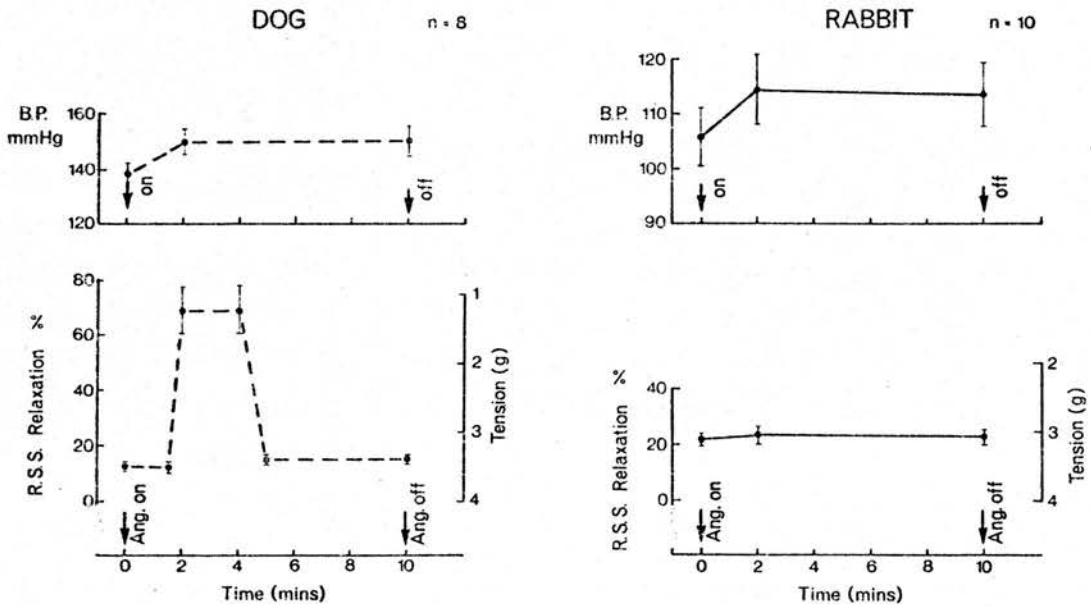
Left panel (upper) represents the mean of arterial blood pressure (B.P.) mmHg of 8 dogs infused with angiotensin (Ang.) 200 ng/kg/min into the aorta for a period of 10 minutes. The left (lower) shows the mean of the changes in tension of the rat stomach strips (R.S.S. Relaxation %) superfused with vena caval blood from the same dogs during the period of angiotensin infusion for 10 minutes.

Right panel (upper) shows the mean of arterial blood pressure (B.P.) mmHg of 10 rabbits infused with angiotensin (Ang.) 200 ng/kg/min into the aorta for a period of 10 minutes.

Right panel (lower) shows the mean of the changes in tension of the rat stomach strips (R.S.S. Relaxation %) superfused with vena caval blood from the same rabbits during the period of angiotensin infusion for 10 minutes.

The arrows point to the time of start and finish of the angiotensin (Ang) infusion.

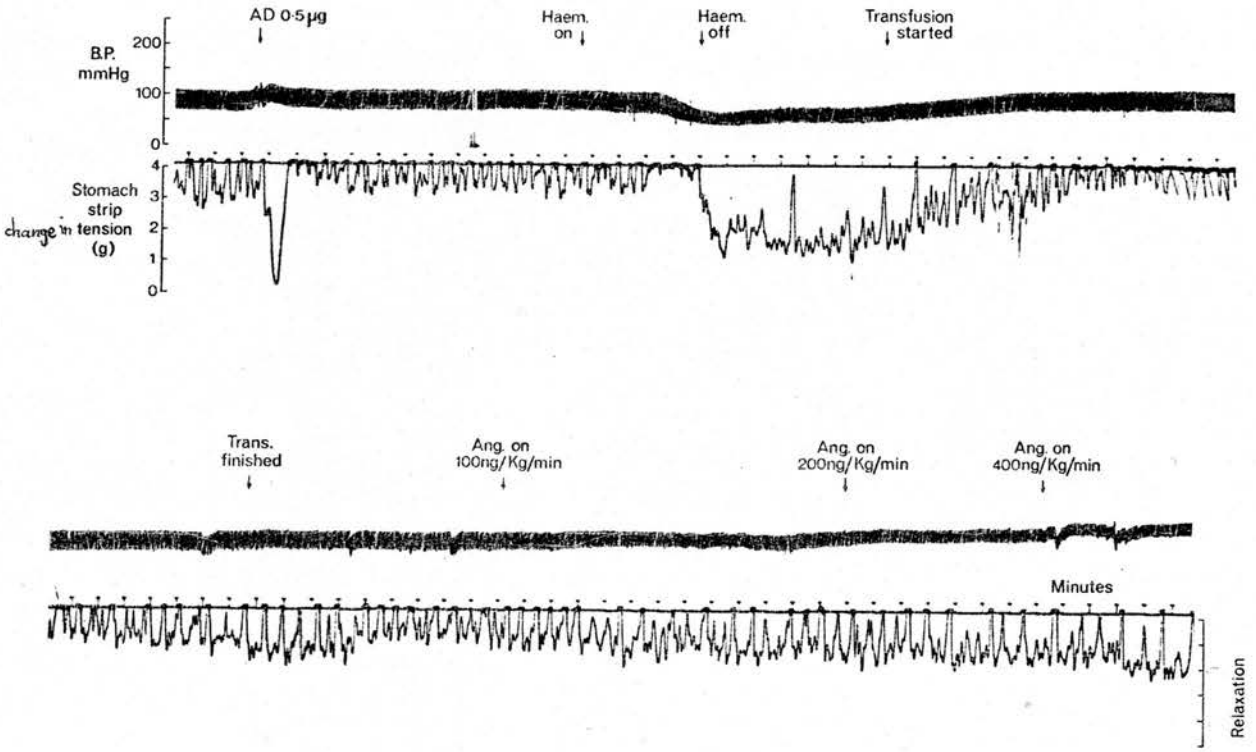
Bars represent standard errors.


DOG
n = 8
B.P. mmHg
160
140
120
on
off
R.S.S. Relaxation %
80
60
40
20
0
Ang. on
Ang. off
1
2
3
4
Tension (g)
0
2
4
6
8
10
Time (mins)
RABBIT
n = 10
B.P. mmHg
120
110
100
90
on
off
R.S.S. Relaxation %
40
20
0
Ang. on
Ang. off
2
3
4
Tension (g)
0
2
4
6
8
10
Time (mins)

Trace 11.

Upper trace shows arterial blood pressure recorded from the carotid artery of a 3·0 kg rabbit anaesthetized with pentobarbitone sodium. A rat stomach strip was superfused with vena caval blood from the same rabbit; when a test dose of adrenaline (AD.) 0·5 μg was injected i.v., the stomach strip relaxed and returned back to normal quickly. Haemorrhage was then performed. At the end of the bleed the muscle strip relaxed and returned back to control base line when the blood was retransfused to the animal.

The lower trace is a continuation of the upper one, when angiotensin (Ang.) 200 ng/kg/min was infused into the aorta, no change in the tension of the strip was noticed. The angiotensin rose to 300 ng/kg/min and showed further increase in blood pressure but no change in stomach strip tension.


AD 0·5 µg
Haem. on
Haem. off
Transfusion started
B.P. mmHg
200
100
0
Stomach strip change in tension (g)
4
3
2
1
0
Trans. finished
Ang. on 100ng/Kg/min
Ang. on 200ng/Kg/min
Ang. on 400ng/Kg/min
Minutes
Relaxation

Trace 12.

A rat stomach strip superfused with vena caval blood from a 12·5 kg dog anaesthetized with pentobarbitone sodium. The arterial blood pressure of the same animal was recorded from the carotid artery. Angiotension (Ang.) 100 ng/kg/min was infused into the aorta for 10 minutes, the arterial blood pressure showed slight increase during the infusion time. The rat stomach strip showed significant increase in relaxation two minutes after the infusion was started and continued relaxing for about two minutes then returned back to control base line before the infusion was finished.

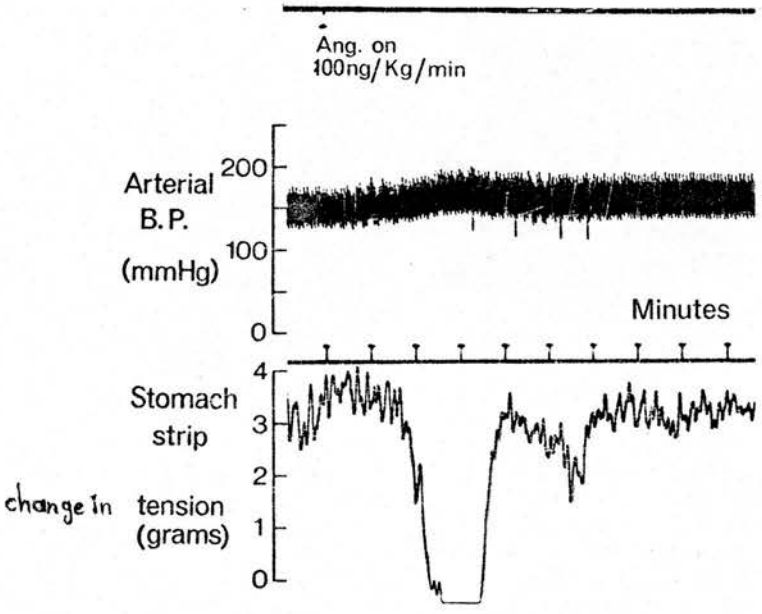

Ang. on
100ng/Kg/min
Arterial
B.P.
(mmHg)
200
100
0
Minutes
Stomach
strip
change in tension
(grams)
4
3
2
1
0

TABLE 13

Control		Ang. infusion 200ng/kg/min. 2 Minutes		Ang. infusion 200ng/kg/min. 10 Minutes	
B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %
82·5	30	85·0	30	85·0	30
95·0	24	105·0	24	100·0	20
120·0	20	125·5	20	122·5	20
85·0	24	92·5	32	92·5	32
130·0	32	140·0	44	140·0	38
100·0	14	105·0	12	110·0	12
110·0	14	115·0	14	115·0	14
130·0	18	145·0	18	135·0	20
110·0	20	130·0	20	130·0	22
95·0	18	100·0	14	105·0	16
105·8 $\pm$ 5·4	21·4 $\pm$ 1·9	114·3 $\pm$ 6·4	22·8 $\pm$ 3·2	113·5 $\pm$ 5·8	22·4 $\pm$ 2·6

Total at the foot of each column represents the Mean $\pm$ S.E.

Table 13. Effect of angiotensin infusion (Ang.) 200mg/kg/min. for 2 minutes and 10 minutes on mean arterial blood pressure (B.P.) and rat stomach strip relaxation (R.S.S. Relax %) in 10 rabbits.

TABLE 14

Control		Ang. infusion 200ng/kg/min. 2 Minutes		Ang. infusion 200ng/kg/min. 10 Minutes	
B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %
132·5	8	140·0	66	140·0	12
135·0	14	145·0	20	140·0	12
145·0	18	160·0	88	160·0	20
150·0	10	165·0	90	175·0	12
127·5	20	145·0	50	140·0	16
157·5	10	170·0	80	170·0	20
137·0	10	145·0	90	145·0	8
125·0	12	130·0	70	135·0	20
138·7 $\pm$ 4·0	12·8 $\pm$ 1·5	150·0 $\pm$ 4·8	69·3 $\pm$ 8·6*	150·6 $\pm$ 5·5	15·0 $\pm$ 1·6

Total at the foot of each column represents the Mean $\pm$ S.E.

Table 14. Effect of angiotensin infusion (Ang.) for 2 minutes and 10 minutes on mean arterial blood pressure (B.P.) and rat stomach strip relaxation (R.S.S. Relax %) in 8 dogs.

* $P < 0{\cdot}001$.

to 200 ng/kg/min after 2 minutes and continued for approximately 10 minutes. The mean arterial blood pressure showed a slight but not significant increase of 8·55 ± 8·39 (S.E. of difference) mmHg after 2 minutes and continued at more or less the same level throughout the period of infusion. No change in the tension of the superfused rat stomach strip was observed at any time during the infusion. In two experiments not included in this group, the dose of angiotensin was increased up to 400 and 800 ng/kg/min again with no significant change in tone of rat stomach strip, suggesting that there was no release of catecholamines from the adrenal medulla (Table 13, Figure 8, Trace 11).

b) Dogs.

In a group of eight dogs the infusion of angiotensin started at 100 ng/kg/min and increased to 200 ng/kg/min after two minutes. Mean arterial blood pressure showed only a slight but not significant increase of 11·31 ± 6·25 (S.E. of difference) mmHg after 2 minutes and it continued at the same level for the remainder of the infusion period. The tension of the superfused tissue changed, markedly, the relaxation increasing significantly ($P < 0·001$) after 2 minutes of the angiotensin infusion. This change of tension was not sustained and tone returned to basal levels after a few minutes in spite of the continuation of the infusion (Table 14, Figure 8 and Trace 12).

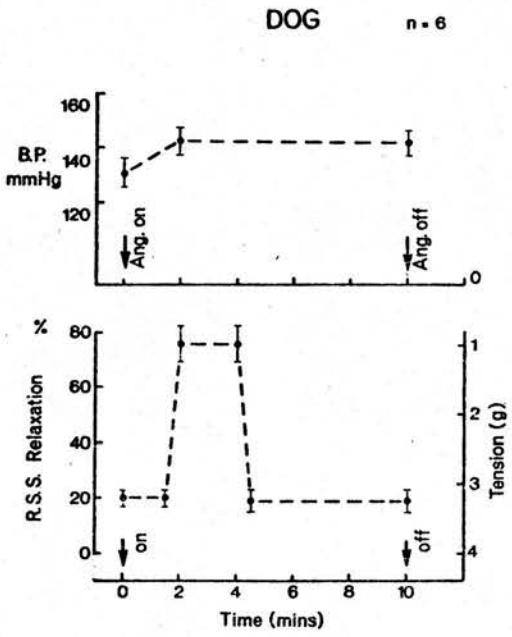
Figure 9.

Left panel (upper) represents the mean arterial blood pressure (B.P.) mmHg of 6 dogs treated with hyoscine methyl bromide 5 mg/kg b.w. Angiotensin (Ang.) 200 ng/kg/min was infused into the aorta for 10 minutes. The left (lower) shows the mean of the changes in tension of rat stomach strips (R.S.S. Relaxation %) superfused with vena caval blood from the same dogs during the period of angiotensin infusion for 10 minutes.

Right panel (upper) shows the mean arterial blood pressure (B.P.) mmHg of 6 dogs treated with hexamethonium bromide 10 mg/kg b.w. Angiotensin (Ang.) 200 ng/kg/min was infused into the aorta for 10 minutes. The right (lower) shows the mean of the changes in tension of rat stomach strips (R.S.S. Relaxation %) superfused with vena caval blood from the same dogs during the period of angiotensin infusion for 10 minutes.

The arrows point to the time of start and finish of the angiotensin (Ang.) infusion.

Bars represent standard errors.


DOG
n = 6
B.P. mmHg
160
140
120
Ang. on
Ang. off
0
%
R.S.S. Relaxation
80
60
40
20
0
on
off
0
2
4
6
8
10
Time (mins)
1
2
3
4
Tension (g)

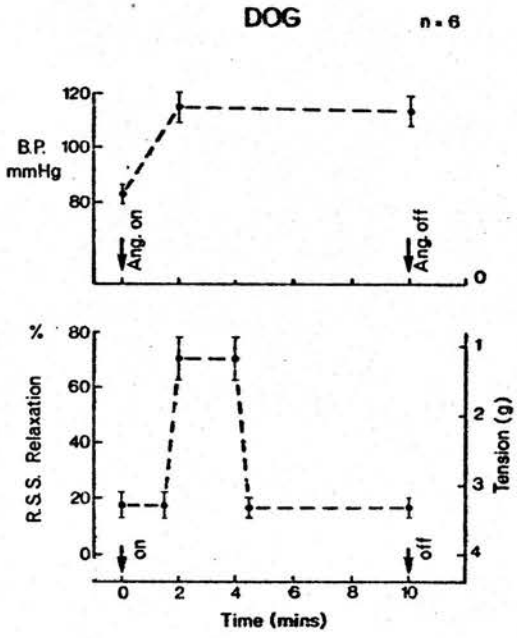

DOG
n = 6
B.P. mmHg
120
100
80
Ang. on
Ang. off
0
%
R.S.S. Relaxation
80
60
40
20
0
on
off
0
2
4
6
8
10
Time (mins)
1
2
3
4
Tension (g)

TABLE 15

Control		Ang. infusion 200ng/kg/min. 2 Minutes		Ang. infusion 200ng/kg/min. 10 Minutes	
B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %
147·5	10	155·5	90	150·0	10
135·0	40	155·0	95	155·0	28
142·5	20	150·0	50	150·0	25
125·0	18	132·5	74	130·0	20
120·0	10	130·0	65	135·0	12
115·0	10	130·0	50	130·0	8
130·8 $\pm$ 5·3	18·0 $\pm$ 4·8	142·2 $\pm$ 5·1	70·7 $\pm$ 7·9*	141·7 $\pm$ 4·6	17·0 $\pm$ 3·5

Total at the foot of each column represents the Mean $\pm$ S.E.

* $P < 0.001$

Table 15. Effect of angiotensin infusion (Ang.) for 2 minutes and 10 minutes on blood pressure (B.P.) and rat stomach strip relaxation (R.S.S.Relax %) in 6 dogs treated with (-) Hyoscin methyl bromide 5mg/kg/b.w.

TABLE 16

Control		Ang. infusion 200ng/kg/min. 2 Minutes		Ang. infusion 200ng/kg/min. 10 Minutes	
B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %	B.P. mmHg	R.S.S. Relax %
85·5	10	135·0	88	130·0	8
75·0	30	100·0	50	100·0	35
95·0	20	125·5	88	125·5	20
88·5	25	112·3	90	115·0	20
75·0	20	105·0	75	100·0	22
80·0	15	110·0	64	105·0	10
83·2 ± 3·3	20·0 ± 2·9	114·6 ± 5·4**	75·8 ± 6·6**	112·6 ± 5·3*	19·2 ± 4·0

Total at the foot of each column represents the Mean ± S.E.

* $P < 0{\cdot}01$ ** P 0·001

Table 16. Effect of angiotensin (Ang.) infusion 200mg/kg/min. for 2 minutes and 10 minutes on blood pressure (B.P.) and rat stomach strip relaxation (R.S.S.Relax %) in 6 dogs treated with Hexamethonium bromide.

Angiotensin infusion was repeated in a group of six dogs after muscarinic blockade with (-) hyoscine methyl bromide and a similar transient relaxation of the superfused tissue was observed 2 minutes after the infusion started ($P < 0.001$) (Table 15, Figure 9, Trace 6).

In a second group of six dogs angiotensin infusion was repeated after nicotinic blockade with hexamethonium bromide. In this group the blood pressure significantly increased 31.46 ± 6.28 (S.E. of difference) mmHg ($P < 0.001$) after 2 minutes of infusion. Starting from lower control value the increase in blood pressure was less than before blockade or with muscarinic blockade. This was accompanied by a significant increase in relaxation of the strip of 55.83 ± 7.19 (S.E. of difference)% from that of control ($P < 0.001$). Again this change in tension was transient and tone returned to basal levels after a few minutes in spite of the continuation of the infusion. The blood pressure remained significantly above normal ($P < 0.001$) during the remaining period of the angiotensin infusion (Table 16, Figure 9 and Trace 8).

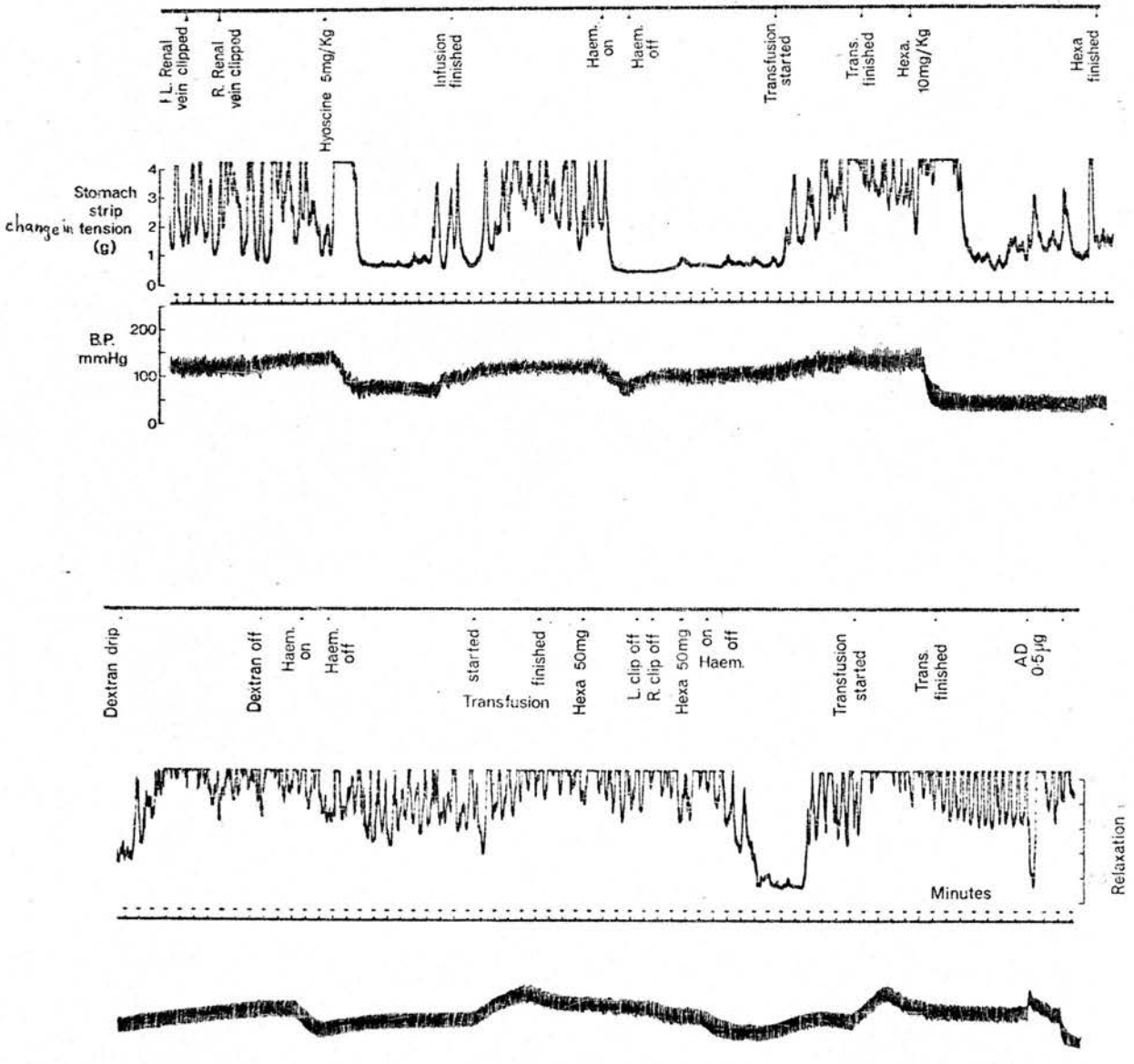
Response of Dogs to Haemorrhage after both Renal Veins Ligation.

As mentioned earlier the angiotensin is released during haemorrhage in dogs and that it is capable of releasing adrenal medullary catecholamine. It was decided therefore to put clips on both renal veins to prevent the renal venous outflow from reaching the

Trace 13.

Upper trace shows a rat stomach strip superfused with vena caval blood from a 12·3 kg dog anaesthetized with pentobarbitone sodium. Arterial blood pressure of the same animal was recorded from the carotid artery. The left (L) renal vein and the right (R) renal vein were clipped, then hyoscine methyl bromide (hyoscine) 5 mg/kg b.w. was infused to achieve muscarinic blockade. Haemorrhage (Haem.) was performed and the rat stomach strip relaxed and continued relaxing until retransfusion of the shed blood started, when it then returned to its control base line. Then hexamethonium bromide (Hexa.) 10 mg/kg b.w. was infused i.v. to achieve nicotinic blockade.

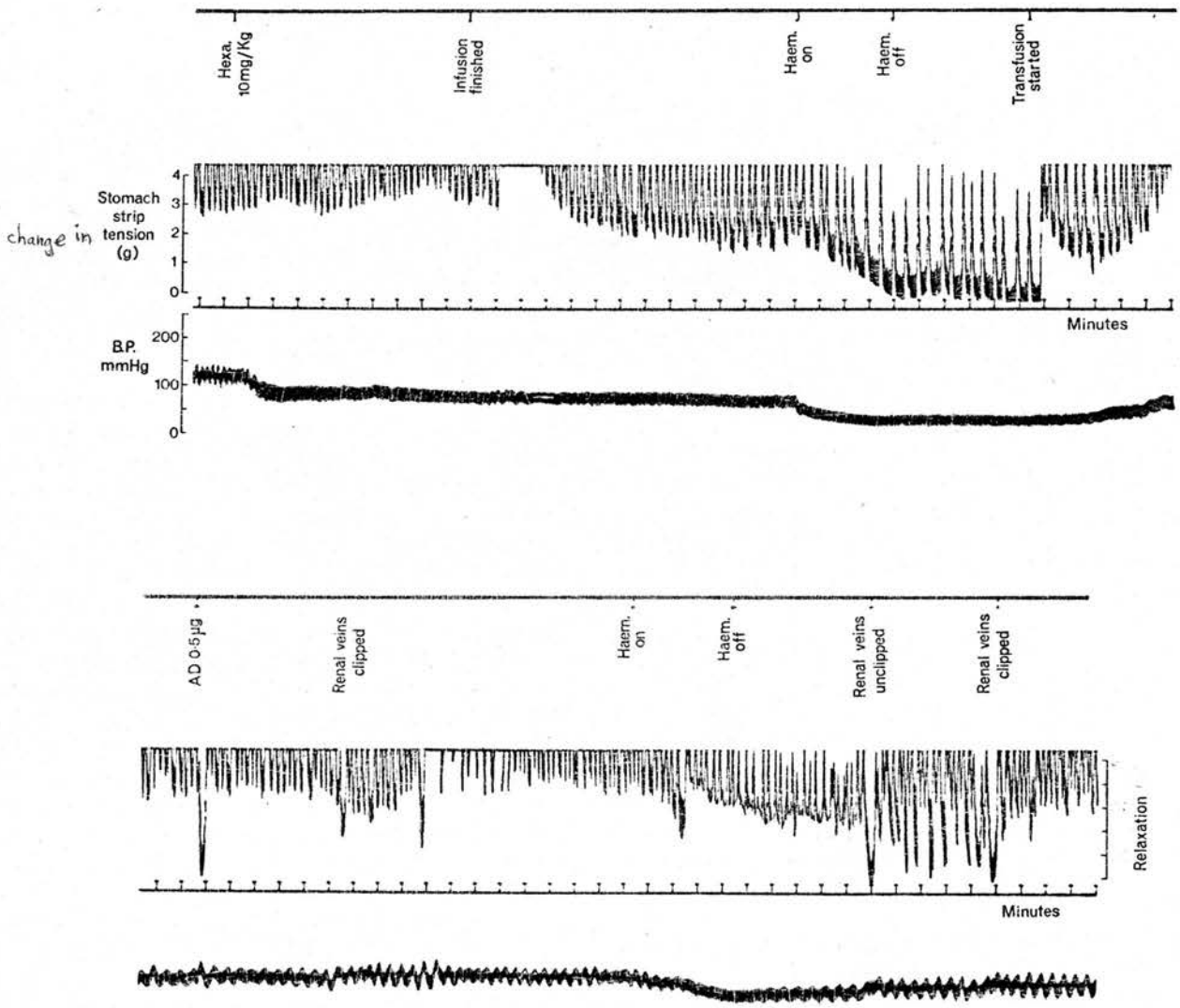
Lower trace shows the continuation of the upper trace, dextran drip was given to stabilize the blood pressure which fell markedly after the infusion of hexamethonium. Haemorrhage was performed which did not cause any change in the tension of the strip. The blood was retransfused to the animal and another dose of hexamethonium (Hexa.) 50 mg was injected i.v., then both left (L) and right (R) renal clips were taken off and another dose of hexamethonium was given. Haemorrhage (Haem.) was then carried out and the rat stomach strip showed significant relaxation which continued only a few minutes and returned to control base line before the shed blood was retransfused to the animal. The sensitivity of the strip was checked with a test dose of adrenaline (AD.) 0·5 μg i.v.


L. Renal vein clipped
R. Renal vein clipped
Hyoscine 5mg/Kg
Infusion finished
Haem. on
Haem. off
Transfusion started
Trans. finished
Hexa. 10mg/Kg
Hexa finished
Stomach strip change in tension (g)
4
3
2
1
0
B.P. mmHg
200
100
0
Dextran drip
Dextran off
Haem. on
Haem. off
Transfusion started
finished
Hexa 50mg
L. clip off
R. clip off
Hexa 50mg
Haem. on
off
Transfusion started
Trans. finished
A.D. 0·5µg
Minutes
Relaxation

Trace 14.

Upper trace shows a rat stomach strip superfused with vena caval blood from a 12·0 kg dog anaesthetized with pentobarbitone sodium. Arterial blood pressure of the same animal was recorded from the carotid artery. Hexamethonium bromide (Hexa.) 10 mg/kg b.w. was infused i.v., after the infusion was finished haemorrhage (Haem.) was carried out and the rat stomach strip showed relaxation and returned back to control base line when the shed blood was re-transfused.

The lower trace shows a continuation of the upper trace, adrenaline (AD.) 0·5 μg test dose was injected i.v. to check the sensitivity of the strip preparation. Then both renal veins were clipped and haemorrhage (Haem) was carried out, no relaxation of the stomach strip was noticed. When the clips were removed from the renal veins (renal veins unclipped) a transient relaxation appeared which disappeared when the renal veins were clipped again.


Hexa. 10mg/Kg
Infusion finished
Haem. on
Haem. off
Transfusion started
Stomach strip tension (g)
change in
4
3
2
1
0
Minutes
B.P. mmHg
200
100
0
AD 0.5µg
Renal veins clipped
Haem. on
Haem. off
Renal veins unclipped
Renal veins clipped
Relaxation
Minutes

circulation. It is well known that angiotensin generates in the blood from the renin released by the kidney so by occlusion of renal venous outflow we are going to prevent any angiotensin from being generated in the circulation.

The renal veins in the dog were relatively easy to isolate and occlude. Both renal veins were embedded in a mass of adipose tissue. Round ended scissors were used in the dissection. A fine thread was passed around each vein by using an aneurysm needle to locate the vein and enable them to be tied or occluded by bulldog clips later on.

In a group of three dogs the renal veins were clipped after blocking the nicotinic receptors with hexamethonium bromide. After a short resting period haemorrhage was carried out. The results showed no significant increase in relaxation of the stomach strip even though the mean arterial blood pressure was significantly decreased ($P < 0{\cdot}001$) at the end of haemorrhage and maintained at this level for 15 - 20 minutes following the haemorrhage. In one of the three dogs the clips were released 5 minutes after the end of the bleed and a significant relaxation happened about one minute after the clip was released. This relaxation disappeared when the renal veins were clipped again (Trace 13 and 14).

DISCUSSION

The Estimation of Plasma Catecholamines.

Fluorimetry alone provides the necessary combination of sensitivity and specificity for the accurate estimation of catecholamines in body fluids. However, while the methods for estimation in urine are relatively uncomplicated the analysis using plasma samples is far from straight forward and requires considerable expertise and a highly sensitive fluorimeter. This is evidenced by the large number of variants of the two basic techniques, the ethylene diamine condensation method (Weil-Malherbe and Bone, 1952) and the trihydroxyindole oxidation method (Eurler, 1948) which are currently available.

The majority of methods in use today require blood samples of at least 5 ml volume, and many require up to 20 ml for reasonably accurate determination of nanogram quantities.

In the present investigation the removal of many blood samples during the course of the experiment would radically affect the overall circulatory condition of the animal, particularly in the case of the rabbit and could also affect angiotensin levels (Hodge, Lowe and Vane, 1966). This effectively precludes the use of these techniques when a continuous assessment of catecholamine changes is to be monitored.

The isolated rat stomach strip preparation was introduced by Vane (1957) as a biological assay

technique for estimating total catecholamines in samples of tissue fluids. In the hands of Vane and his co-workers it was developed into a highly sensitive method capable of detecting nanogram quantities of adrenaline and noradrenaline (Armitage and Vane, 1964; Regoli and Vane, 1964) in blood and other body fluids. Further development, by Vane (1964) led to the introduction of the superfused stomach strip where the tissue is continuously bathed by the circulating blood of the experimental animal. The volume of the extracorporeal circuit is crucial particularly for smaller animals. Hall and Hodge (1971) reduced it to 10 - 15 ml while estimating both catecholamine and angiotensin levels. Our own circuit had a volume of between 7·5 and 10 ml and was primed with saline or dextran-saline.

Flow rates are also important. In his experiments on dogs, Vane regarded flow of 12 - 15 ml/min as optimal for maximal sensitivity of the perfused tissues. For dogs flow rates of 10 - 12 ml/min were used in the present work. However, such flows were not possible with rabbits, the maximal rates attainable being 7 ml/min in large animals with 5 ml/min for most experiments. Sensitivity of the perfused tissues never reached the very high levels obtained by Vane and his co-workers, but were quite adequate to detect released hormones under the conditions of the present experiments.

Some early experiments gave disappointingly low levels of catecholamine release during haemorrhage. In these

animals blood for perfusion was drawn from a carotid artery. From the work of Watts and Westfall (1964) it would seem that considerable breakdown of catecholamines might occur in the tissues between their release into the adrenal vein and their entry to the extracorporeal circulation. In rabbits, cannulation of the adrenal veins is impossible on the right side and extremely difficult on the left. In any case flow rates would be too small to maintain a viable superfusion circuit. Blood for the external circuit was therefore collected from a cannula entering the femoral vein and pushed into the vena cava until its tip lay centrally to the right (the higher) adrenal vein.

In dogs collection of adrenal venous blood is more readily attained, but to keep the experimental conditions similar in both animals and to reduce surgical interference the same technique was used with wider bore cannulae.

Some of the early superfusion attempts with the rabbit were very disappointing in the lack of response of the stomach strip to injection of known doses of catecholamine. Particularly noticeable was the lack of resting tone and it was further observed that the stomach strip of a mature female rat was sometimes more effective than that of the immature female or the male.

Observations made by Lloyd (1959a) showed that in female rats, oxytocin, which was usually dilator to the mesenteric vessels, and without effect on the blood pressure, had a constrictor action on the blood vessels and pressor effect on the blood pressure during the period

of natural oestrus. The same effects were observed temporarily after administration of stilbestrol and progestrone. Even in male rats where oxytocin dilated the mesenteric vessels, after the administration of stilbestrol blood vessel vasoconstriction and pressor responses of blood pressure were noted. During the second half of pregnancy in rats oxytocin had a pressor action (Lloyd, 1959b) and this increased as pregnancy progressed while in the first half of pregnancy oxytocin was without effect. The intravenous or intra-arterial injection of oxytocin caused an increase in the rate of blood flow through the limb of normal male and female dogs (Lloyd and Pickford, 1952) while after treatment of dogs with an oestrogen or giving intravenous oestrogen during acute experiments, similar doses of oxytocin caused a reduction in the rate of blood flow. On the other hand in human beings (Haigh, Kitchin and Pickford, 1963) confirmed that intra-arterial oxytocin caused an increase in blood flow at the site of injection in normal men and women, while 40 - 50 minutes after intra-arterial oestrogen, oxytocin caused a reduction in blood flow at the site of injection, and flow through the other, control, hand was not affected. They suggested that the reduction in hand blood flow after oestrogen is due to direct vasoconstrictor effect of oxytocin.

It was therefore decided to use female rats and they were injected with stilbestrol 0·1 mg/kg body weight in oil base subcutaneously 18 hours before the experiment.

This ensured that stomach strips gave a sensitive response to catecholamine regularly, largely due to the better initial resting tone of the preparation.

The only disadvantage the workers demonstrated while assaying substances by isolated smooth muscles was the lack of oxygen which results mostly in loss of tone reducing the sensitivity of the tissues to some drugs. The substitution of nitrogen for oxygen through the bath resulted in complete mechanical inactivity of isolated rabbits intestine 3 - 4 minutes after exposure to nitrogen (West, Hadden and Farah, 1951), and also anoxia was shown to abolish the tonus change so with acetylcholine 0·5 - 2·5 µg/l only a single contraction appeared, lasting from 5 - 15 seconds while normal aerobic response to acetylcholine may be considered a biphasic response. This observation confirmed the work of Gross and Clark (1923) which showed that the effect of adrenaline and noradrenaline were diminished by lack of oxygen. Born (1956) also confirmed these findings using the taenia coli muscle of a Guinea-pig. Lack of oxygen made the taenia coli lose spontaneous tension and activity. Further confirmation of the effect of hypoxia come from the work of Blair and Clark (1956) who show that oxygen deprivation at 1°C and 3°C for 19 - 24 hours rendered the intestine unresponsive to substance P which normally produced a contraction. Day and Vane (1963) showed reduction in the response of Guinea-pig ileum to 5-hydroxytryptamine which is not due to tachyphylaxis and Carrier, Walker and Guyton (1964);

Smith and Vane, (1966) showed that resting tension of various isolated intestinal and smooth muscle preparations varied directly with the pO_2 of the stream of blood used to bath them and the effects could not be antagonized by specific pharmacological antagonists. They suggested the reaction of the isolated organs to catecholamines and 5-HT were increased by the higher pO_2. They also investigated several mechanisms by which oxygen tension could influence the resting tension of smooth muscle. One would be direct interaction between oxygen and an "oxygen receptor". Another possibility was the formation or release of substances, within the tissue, which could induce contraction or relaxation when exposed to high and low oxygen tension respectively. A third possible mechanism is that the provision of energy necessary for the maintenance of muscular tone depends upon an enzyme controlled process, the rate of reaction of which is limited by the oxygen available in the surrounding fluid. In this work lack of oxygen was reduced by allowing the animal to breath 50% oxygen in air. However, in the rabbits particularly, the venous perfusate was sometimes very desaturated. Our own experiments unlike those of Smith and Vane showed no effect on the muscle tone and contraction until the pO_2 level had fallen below 20 mmHg. The venous perfusate was monitored regularly during haemorrhagic episodes when pO_2 tended to be at their lowest and any responses where pO_2 fell below 30 mm were discounted. Furthermore, the relaxation of the fundus strip was

abolished during haemorrhage in rabbits in which their adrenal veins were clipped. This gave no evidence to the effect of the lack of oxygen in this investigation.

Circulatory and Respiratory changes following Haemorrhage.

1. Circulatory responses following haemorrhage.

a) Heart Rate. One of the more important compensatory responses to haemorrhage is the increase in heart rate induced by sympathetic activity (Cannon, 1923; Sarnoff and Mitchell, 1962).

Our own experiments in anaesthetized rabbits showed only a marginal increase in heart rate of 9 beats/min during the first minute of bleed. This was a transient effect and heart rate fell to lower than control at 30 minutes after the end of the bleed, this depression might be due to the development of hemodilution due to spontaneous reabsorption of extravascular fluid (Korner, 1971). Chalmers, Korner and White (1967) showed a marked increase of 30 beats/min in heart rate during haemorrhage, but it must be noted that their rabbits were unanaesthetized. The effect of the anaesthetic may be of importance since the resting heart rate of our rabbits was high before commencing any bleed procedures.

Observations made by Chein (1958) have shown that increase in heart rate in anaesthetized dogs after haemorrhage was abolished in sympathectomized dogs.

In our dog experiments like those of Chein (1958) the heart rate increased markedly (18·1 beats/min) during

the first minute of bleed and increased significantly (30 beats/min) at the end of bleed. The increase of heart rate during haemorrhage was not affected by muscarinic blockade but was abolished by the administration of hexamethonium to the animal before haemorrhage. Unlike that of the rabbit, the heart rate of the dog did not show the depression below control at 30 min after bleed, this may be the animal maintaining the venous return by the redistribution of the remaining vascular volume subsequent to splenic contraction and possibly hepatic and venous contraction (Haddy, Scott and Molnar, 1965).

b) Effect of haemorrhage on blood dilution. The restoration of blood pressure towards control values after haemorrhage in both rabbits and dogs suggested the entrance of tissue fluid into the circulation. Transfer of tissue fluid after haemorrhage has been reported by many workers (Cannon, 1923). Some, like Halpern, Benacerraf and Briot (1952) showed that in the early stage of haemorrhage in adrenalectomized rats, a slight haemoconcentration, corresponding presumably to the mobilization of blood reserves occurred, but in the later stages there is a slight haemodilution, caused by absorption of fluid from the extracellular compartment. The dilution of blood did not require 2 - 3 hours for a significant change to be observed as was the case in unanaesthetized rabbits (Chalmers, Korner and White, 1971). In our rabbit experiments significant dilution indicated by reduction in both haemoglobin concentration ($P < 0{\cdot}01$) and packed cell volume

($P < 0{\cdot}05$) was obvious only 30 minutes after haemorrhage.

With dogs some conflict of evidence is noted. Guyton, Batson and Smith (1951) have shown that interstitial fluid enters the circulation very slowly after a rapid massive haemorrhage in normal dogs as determined from the hematocrit. Other workers indicate a very rapid transfer of extracellular fluid in this species (Haddy, Scott and Molnar, 1965). In our dog experiments dilution of blood had not taken place 30 minutes after haemorrhage as determined by increase in haemaglobin concentration and P.C.V. This might be due to the large number of erythrocytes released from the spleen in the circulation during the early stage of haemorrhage. The spleen is extremely large in anaesthetized dogs, particularly when barbiturates are used, and contraction during haemorrhage might well mask any plasma dilution due to entry of extra cellular fluid into the circulation. In many class experiments in the department (A.L. Haigh, personal communication) blood dilution has been observed one hour after bleeding. This is in agreement with the findings of Guyton, Batson and Smith (1951). Our experiments also agree with those of Haddy, Scott and Molnar (1965) who suggested that splenic discharge in haemorrhage is induced by baroceptor stimulation leading to sympathico adrenal discharge.

c) Effect of Haemorrhage on pH, p_aCO_2 and p_aO_2.

The experiments with rabbits showed a significant increase in arterial pCO_2 ($P < 0{\cdot}01$) and decrease in pH value ($P < 0{\cdot}05$), but with no significant change in the p_aO_2 values. These results suggested arterial hypercapnea and acidosis. In the dog experiments on the other hand no significant changes in pH, pCO_2 and pO_2 occurred. These results are in agreement with those of Crowell and Guyton (1961; 1962) who did not observe significant change in oxygen consumption in haemorrhagic shock in the dog. A possible explanation of the above results has been put forward by Korner (1971). He states that the rabbit is a small-lung species with a lung/body weight ratio of about 0·5% whereas the dog is a large-lung species with a lung/body weight ratio of about 1% and much greater capacity to increase ventilation during hypoxia.

2. Effect of Haemorrhage on Respiration.

The results in this investigation in anaesthetized rabbits agree with those of Chalmers, Korner and White (1967) for the unanaesthetized rabbit. The minute volume is unchanged while tidal volume showed a significant decrease during haemorrhage together with an increase in the respiratory rate.

In dogs there was a significant increase in minute volume due mainly to the increase in the respiratory rate.

Possibly the large size of the lung of the dog combined with the increase in respiration rate results in an

increased minute volume even though tidal volume was slightly decreased at the end of haemorrhage.

3. Adrenal Medullary Responses to Haemorrhage.

a) Effect of Haemorrhage on Catecholamine Release.

Dogs:

The investigation of Bedford (1917) demonstrated increase of adrenaline in the vena cava of anaesthetized dogs during haemorrhage and this increase was accompanied by hyperactivity of the adrenal medulla. Other workers have also shown increased levels of plasma adrenaline after blood pressure had been lowered to a level leading to shock (Watts, 1956; Watts and Bragg, 1957). Both adrenaline and noradrenaline increased during haemorrhagic hypotension in anaesthetized dogs (Poole and Watts, 1959; Millar and Benfey, 1958) and the increase is in adrenaline rather than noradrenaline (Walker, Sherefettin Zileli, Reutter, Shoemaker, Friend and Moore, 1959: Watts and Westfall, 1964). Our own experiments on anaesthetized dogs was in general in accordance with the above observations. Catecholamine secretion increased after lowering blood pressure but unlike Bedford (1917), in our experiments prolonged hypotension was not required before release of catecholamines occurred. In most of the experiments the catecholamine level in the vena cava showed a marked or significant increase 3 - 4 minutes after the start even though the blood pressure was not by then reduced to the low levels described by Watts (1956).

The results show better agreement with those of Watts and Westfall (1964) where increase of circulating catecholamines in dogs was obtained with blood pressure lowered below 80 mmHg. At the end of the bleed when the mean blood pressure had reached 50 - 60 mmHg there was a significant increase of catecholamine in the vena caval blood ($P < 0.001$) as judged by the marked relaxation of the rat stomach strip. Although blood pressure recovered considerably in most experiments during the resting period of 20 - 30 minutes after the end of haemorrhage, catecholamines release continued at the same level of output until the shed blood was returned to the animal and blood pressure returned fully to control levels. This is in agreement with Greever and Watts (1959) who confirmed the findings of Walker et al (1959) and suggested that blood volume changes may be more important than alteration in arterial blood pressure in governing adrenal medullary secretion.

The striking increase in catecholamine release throughout the period of the bleed as indicated by relaxation of the stomach strip might be due to a long lasting effect on the excised tissue. This is unlikely for two reasons, firstly, the stomach strip returned to its control base line when only a little of the shed blood was retransfused in the circulation. Secondly, the early experiments using rabbits blood from the carotid artery perfused over the stomach strip had much less effect than vena caval blood.

This suggests a very rapid breakdown of most of the catecholamine released as it passed through the body tissues. Moreover, the results of perfusing vena caval blood from the rabbit over the stomach strip during haemorrhage were very different from those obtained in the dog. The strip did not exhibit continuous relaxation and in most experiments its tone had returned back to control base line before the blood retransfusion started. Further observations of Poole and Watts (1959); Watts and Westfall (1964) may be of interest here. They suggested that adrenaline is rapidly inactivated when it is brought into intimate contact with the tissues, and thought that the first increase in catecholamine release following haemorrhage is due to the release of the substance stored in the chromaffin cells.

In the later stages of haemorrhage release is due to continuous reflex activation of the adrenal gland to secrete further catecholamine. There was no evidence of any reduction in arterial pH and no significant reduction of p_aCO_2 in the dog experiments carried out in the present study, this tends to reduce the likelihood that metabolic acidosis stimulates the adrenal gland to secrete larger amounts of adrenaline or decrease in its destruction during the late stage of haemorrhage as suggested by Darby and Watts (1964).

Rabbits:

The results of our rabbit experiments are unlike those of the dog. In this species we found that prolonged

lowering of blood pressure is needed before any significant release of catecholamine can be detected by the smooth muscle used for the assay. Blood pressure had to be reduced to 50 - 60 mmHg before any detectable relaxation in the stomach strip was apparent. At the end of the bleed or one minute after, a significant release of catecholamine started which was of a transient nature. Sometimes the tone of the rat's stomach strip returned to its resting baseline well before the retransfusion of the shed blood started. This may be due to incontinuous activation of the adrenal medulla. In our early experiments it was a cause of some concern that hypoxia might be the cause of the relaxation of the superfused tissue. Two kinds of experiments were carried out to investigate that the relaxation was due to catecholamine and not an artifact due to hypoxia. In several experiments rabbits were allowed to breath 7% oxygen in N_2 and even though the oxygen saturation of the blood was markedly reduced, the tension of the perfused strip was virtually unaffected until the pO_2 level of the vena caval blood reached levels of about 15 mmHg. In all subsequent haemorrhage experiments venous pO_2 always exceeded 20 mmHg and it was usually in the range 25 - 30 mmHg. In a second group of 3 rabbits the adrenal veins were clipped off before bleed commenced. No evidence of relaxation in the stomach strip was noticed after the bleed although the blood pressure was reduced to the same levels as before (50 - 60 mmHg). Those experiments appear to substantiate the relaxation of stomach strip

as being due to an increase of circulating catecholamine released from the adrenal medulla during haemorrhage. It seems very likely that the release is due to sympathetic activity following the reduction in total blood volume, detected mainly by arterial type B receptors (Milnor, 1974).

Our investigation in rabbits revealed a significant decrease in arterial pH and pCO_2 which might suggest that the increased catecholamine release is due to stimulation of the adrenal medulla by acidosis directly or by reflex chemoreceptor stimulation.

The Effect of Muscarinic Blockade on Catecholamine Release.

Dogs:

Administration of muscarinic receptor blockade (-) hyoscine methyl bromide did not alter the amount or duration of the release of catecholamine judged by the continuous relaxation of rat stomach strip over all the period following the end of the bleed. These results suggest no evidence of measurable population of muscarinic post ganglionic receptors controlling the release of catecholamine from the adrenal medulla. The existance of muscarinic receptors in sympathetic ganglia has been studied by many workers (Trendelenburg, 1966; Brown, 1967; Flacke and Gillis, 1968). Muscarinic antagonists have been shown to stimulate catecholamine release from the adrenal medulla of the dog (Critchley, Tibenham, Ungar, Wait and West, 1975); the action of these secretagogues is inhibited by muscarinic antagonists such as atropine. Henderson and Ungar (1977)

showed that in canine adrenal medulla there exists a population of muscarinic receptors.

Rabbits:

In rabbits as in the dog, the administration of hyoscine methyl bromide had no effect on the release of catecholamine from the adrenal medulla during a subsequent bleed and again suggests no major population of muscarinic post ganglionic receptors.

The Effects of Nictotinic Receptor Blockade on Catecholamine Release.

Dogs:

Administration of hexamethonium bromide in the dog to block the nicotinic receptor sites, produced results unlike those of Walton, Richardson, Walton and Thompson (1959). Blood levels of catecholamines at the end of bleed were reduced and were less than that during haemorrhage prior to blockade or in haemorrhage after muscarinic blockade. Moreover, the release was transient and not continuous over all the period. It could be abolished if both renal veins were ligated together with hexamethonium administration before the bleed started, but the ligation of the renal veins alone or hexamethonium alone did not abolish the release of catecholamine. It was this finding that drew our attention toward angiotensin II because of the known action of the renin-angiotensin complex in causing release of adrenal catecholamines (Feldberg and

Lewis, 1964, 1965; Staszewska-Barczak and Vane, 1965, 1967; Peach, Cline and Watts, 1966). Also, it is known to be released during haemorrhage in many species. This part of the investigation will be discussed further later on.

Rabbits:

After the administration of hexamethonium bromide in the rabbit unlike dogs, catecholamine release from the adrenal medulla was abolished entirely during and after haemorrhage in which the blood pressure was significantly reduced. Since the rabbit responded before blockade, it suggests that the adrenal medulla of the rabbit contains only nicotinic ganglionic receptors. These results were very interesting in that, unlike the dog, ganglionic blockade totally abolished catecholamine release. It was decided therefore to study further the role of angiotensin in catecholamine release during haemorrhage in these two species.

Effect of Carbachol Injection on Catecholamine Release.

The secretory cells of the adrenal medulla are embryologically derived from nervous tissue and are analogous to postganglionic neurons (Guyton, 1977). In both species studied our experiments suggest that muscarinic post ganglionic cells do not exist, specially as hyoscine methyl bromide did not diminish the release of catecholamine during haemorrhage in both species while hexamethonium did.

Carbachol which is a parasympathomimetic drug has an

action practically equivalent to the effect of acetylcholine at the nerve terminals in the adrenal glands which cause secretion of adrenaline and noradrenaline (Koelle, 1975). Carbachol increases the tone and amplitude of contraction of the gastro-intestinal system. In our experiment injection of a single dose (1 mg) of carbachol resulted in a significant release of catecholamine from the adrenal medulla in both rabbit and dog. The same dose was repeated after administration of hyoscine methyl bromide which blocks the muscarinic action of carbachol. The same significant release of catecholamine was observed. Injection of carbachol in the same dose to both rabbit and dog after the administration of hexamethonium bromide which blocks the nicotinic action of carbachol completely abolished the release of catecholamine in both species. These results are further evidence that most of the receptor population in the adrenal glands are nicotinic in both rabbits and dogs.

Effect of Haemorrhage on Angiotensin Blood Level.

Rabbits:

Renin has been assayed in the plasma of rabbits (Lever and Robertson, 1964; Ryan, McKenzie and Lee, 1968) under normal conditions, and has been extracted from rabbit kidneys treated with alcohol many years previously (Pickering and Prinzmetal, 1938). Some workers have suggested organs other than the kidney, such as the uterus, (Ferris, Gorden and Mulrow, 1967a, 1967b) as a source

of renin in the rabbit. The enzyme isolated produced a vasopressor substance after incubation with renin substrate (Ryan and Ferris, 1967) and they suggested that the product was probably angiotensin I. But the finding of Lumbers (1973) provided evidence that the kidneys of the female rabbit as well as the male is the major source of plasma renin. Rabbits made hypertensive by the application of a renal artery clip showed significant increase in renin level in the plasma (Lever and Robertson, 1964). The superfused rat's ascending colon treated with propranolol (Regoli and Vane, 1964a) was used in investigating the changes in blood angiotensin level during haemorrhage in the dog. McKenzie, Lee and Cook (1966) showed that when rabbits were bled severely until their blood pressure fell to 25 - 40 mmHg, there was a rapid increase of arterial plasma renin activity. In our experiments, unlike those of McKenzie et al., the blood pressure fell to a minimum of 50 - 60 mmHg at the end of the bleed. In our experiments in rabbits neither blood from the vena cava nor carotid artery cause any contraction of the rat colon during haemorrhage. These findings seem to indicate that there was no increase of blood levels of angiotensin, probably due to a lack of increased renin secretion from the kidney. Either the method is insensitive to the amounts of angiotensin released which was unlikely, or blood pressure has to be reduced to even lower, almost fatal, levels to produce secretion or release of angiotensin is not an important factor in

immediate fluid volume changes in haemorrhage.

Dogs:

Reduction of mean arterial pressure was found not to be a necessary condition for increased renin secretion (Kohlstaedt and Page, 1940a). In normal isolated kidney of the dog perfused with blood renin appeared to be produced when pulse pressure and blood flow are reduced by constricting the renal artery. Haemorrhage has been reported to cause an increase in renin secretion (Sapirstein, Ogden and Southard, 1941; Hamilton and Collins, 1942). Angiotensin blood levels in dogs have been shown to be increased during haemorrhage (Regoli and Vane, 1964b), these workers demonstrated contraction in rat colon within 2 minutes after reducing the blood pressure by haemorrhage. They also provided evidence that the contraction is due to angiotensin activated by the release of renin from the kidneys since contraction disappeared after nephrectomy. Our results confirm their findings and show both the increase in angiotensin blood level after haemorrhage and the abolition of colon contraction after clipping the renal veins.

Our results, unlike those of Regoli and Vane (1966) which showed appearance of the angiotensin in the circulation of the dog after small losses of blood, required a moderate haemorrhage to produce significant colon contraction indicating angiotensin release. This may have

been due to the fact that the sensitivity of our colon preparations was never as great as theirs. The results do agree with those of Hall and Hodge (1971) who showed an increase in blood angiotensin levels after haemorrhage in dogs with the appearance of angiotensin preceding that of catecholamine.

Effects of Angiotensin Infusion.

Rabbits:

Probably the work of Feldberg (1941) is important in helping to explain our results in rabbits. He showed that bee and cobra venoms cause a long lasting output of the adrenaline from the adrenal medulla when injected in the central stump of the coelic artery of a cat. He attributed their action to the formation of lysolecithin in the adrenals. Lysolecithin was found to cause an output of adrenaline similar to that produced by venom in the cat but in the rabbit it has either no effect or only slight and inconsistant secretory action on the adrenal medulla. The importance of this work to our own is that it shows differences in the sensitivity of the adrenal medulla in different species. Our results show that the adrenal medulla of the rabbit is not sensitive to the aortic infusion of angiotensin II even in high doses such as 400 - 800 ng/kg/min while angiotensin was reported to be a strong catecholamine releaser from the adrenal medulla in the dog and cat (Feldberg and Lewis, 1964, 1965; Staszewska-Barczak and Vane, 1965, 1967; Peach, Cline and

Watts, 1966) and Guinea pig (Piper, Collier and Vane, 1967; Piper and Vane, 1967). The bovine adrenal medulla was reported not to be sensitive or hardly sensitive to angiotensin II either directly or indirectly (Comline, Silver and Sinclair, 1968).

Dogs:

The results in dogs, unlike those of the rabbit, showed that a significant release of catecholamine started 1 - 2 minutes after the start of infusions of 200 ng/kg/min angiotensin into the aorta central to the renal artery. The release continued for 2 - 3 minutes but was not maintained for the whole period of the infusion, indeed its effect dropped very rapidly. Our results confirm those of Staszewska-Barczak and Vane (1965) and Feldberg and Lewis (1965) who showed that in neither dog nor the cat was the effectiveness of the angiotensin decreased by ganglion blocking agents such as hexamethonium bromide. In the present investigation angiotensin infusions (200 ng/kg/min) in dog were given after muscarinic blockade with hyoscine methyl bromide or after nicotinic blockade with hexamethonium in an attempt to modify the release of catecholamine. Neither of the two blocking agents decreased the releasing effect of angiotensin on the adrenal medulla. These findings are highly suggestive that angiotensin has a direct action on the chromaffin cells causing them to release catecholamine.

Staszewska-Barczak and Vane (1965) showed that angiotensin (2 - 50 μg/kg) caused an initial burst of adrenaline

secretion which rapidly declined during the infusion and within 5 - 10 minutes the secretion stopped; and additional injection of angiotensin produced no further response. Our results fully confirm this finding except that the dose used was much smaller (200 ng/kg/min). Our doses were however larger than those used by Peach, Cline and Watts (1966) who infused 25 - 100 ng/kg/min via the femoral vein. This may be due to the fact that they were estimating released catecholamine by the sensitive fluorimetric technique involving the trihydroxyindole oxidation reaction described by Robinson and Watts (1965). However, this technique requires large blood sample (20 ml) for each analysis. This precluded its use in the present investigation where even one blood sample might cause secretion of renin, increase the circulating angiotensin and cause release of catecholamine from the adrenal medulla.

It would appear that important species differences exist so far as the hormonal responses to haemorrhagic hypotension is concerned. Hall and Hodge (1971) using the same techniques as those used in this study have shown that the rate of haemorrhage is particularly important in catecholamine release in the dog but not the cat, whereas angiotensin release was unaffected. In the present study rate of haemorrhage was kept constant at what would roughly correspond to what previous workers have described as slow. Differences in the release of angiotensin during haemorrhage are described, as are differences in the catecholamine releasing effects of angiotensin.

What the significance of these differences are so far as the compensatory responses to haemorrhage are concerned, have not so far emerged. It would appear that the adrenal medullary responses in the rabbit in particular, play little part in the immediate cardiovascular adjustments to haemorrhage. The same would appear to be true for angiotensin. In the dog, however, both angiotensin and catecholamines may have a role in the immediate readjustments after haemorrhage as well as a long term effect on fluid volume and metabolism. One might suggest tentatively that the differences described might be involved in the known vulnerability to cardiovascular collapse of the rabbit following a large haemorrhage, compared with the very robust reactions of most dogs.

SUMMARY AND CONCLUSIONS.

From the foregoing results one can conclude that the superfused rat stomach fundic strip provides a sensitive method for continuously recording the release of catecholamines into the circulation. Injection of the animal with oestrogen subcutaneously 18 hours before the experiment gave a reliable and sensitive preparation for the assay of catecholamines released from the adrenal medulla during haemorrhage. Rat ascending colon soaked in a propranolol, (10^{-6} g/l) for half an hour before use provided a sensitive assay preparation for angiotensin generated during haemorrhage.

The rabbit catecholamine released from the adrenal medulla increased at the end of the period of haemorrhage which took 5 minutes to complete, at which time the blood pressure was lowered to about 50 - 60 mmHg. The release of catecholamine is not continuous and decreased after the haemorrhage stopped. In some experiments catecholamine level had returned to control levels before the re-transfusion of the shed blood commenced, although some intrinsic compensation and rise of arterial blood pressure had occurred. The blood pressure recovered slightly during the 30 minutes resting after the bleed, partly due to sympathetic influence, but also due to the rapid entrance of tissue fluid into the circulation as shown by decrease in both packed cell volume and haemoglobin concentration. Heart rate did not increase significantly

in this species during haemorrhage and fell below control levels 30 minutes after haemorrhage. This might be related to the increase of plasma volume due to entry of extra-cellular fluid. This small rise in heart rate at the beginning of haemorrhage is probably related to the high resting heart rates in these anaesthetized animals.

The release of catecholamine during haemorrhage in the rabbit was not reduced by muscarinic blockade but was abolished by nicotinic blockade which indicates most of the receptor population are nicotinic, a finding supported by the experiments with carbachol.

No evidence of increase in angiotensin generation during or after haemorrhage suggests that no significant release of renin from the kidney of the rabbit occurs during or 30 minutes after the haemorrhage procedures, during which arterial blood pressure was reduced to 50 - 60 mmHg. Angiotensin infusions into the aorta in 200, 400 and even 800 ng/kg/min did not give any evidence of releasing catecholamines from the adrenal medulla nor did discrete injections of angiotensin. This was taken as evidence that in the rabbit angiotensin does not act as a catecholamine releaser from the adrenal medulla.

The above findings suggest that angiotensin neither releases catecholamine in significant amounts during haemorrhage, nor does it play any part in catecholamine release during haemorrhage in the rabbit.

In dogs, catecholamine release from the adrenal

medulla commences as blood pressure falls and before the end of haemorrhage in most experiments when blood pressure was 50 - 60 mmHg. Low levels of blood pressure did not have to be sustained for the increase in catecholamines to occur. Release was continuous over the whole period (30 minutes) after the bleed, in spite of the compensatory increase in arterial blood pressure. It returned to normal level when retransfusion of the shed blood began.

of the dog

The heart rate increased during haemorrhage especially towards the end and returned to control level within 30 minutes after haemorrhage. The increase in catecholamines released during haemorrhage was not affected by muscarinic blockade. In animals treated with hexamethonium however, catecholamine release increased during haemorrhage but not to the same level as during a bleed prior to blockade. Moreover, the output of catecholamine was a transient one persisting for only a few minutes in most of the animals studied and returning back to control levels before retransfusion started. Ligation of the renal veins together with prior administration of hexamethonium totally abolished the release of catecholamine after haemorrhage.

in the dog

Angiotensin blood level increased markedly after haemorrhage and only returned to control levels after retransfusion of the shed blood. Infusion of angiotensin in 200 ng/kg/min significantly increased the release of catecholamine from the adrenal medulla in a transient burst which persisted for only 2 - 3 minutes and not for

the whole period of the infusion. It resembled the responses noted during haemorrhage after ganglionic blockade with hexamethonium.

The above results suggest that the adrenal medulla of the dog is very sensitive to angiotensin and that angiotension liberation during haemorrhage precedes the release of catecholamine in the dog and could play an important role in the initial release of catecholamine into the circulation during haemorrhage in this animal.

The species differences noted are discussed in the context of the cardio vascular control in hypotensive states.

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APPENDIX

Specificity of the rat stomach strip assay:-

Vane and his co-worker (Armitage & Vane, 1964; Vane, 1964; Vane, 1969) showed that the relaxation of the rat stomach strip is a specific action of catecholamines and most of the other substances e.g. 5-hydroxytryptamine (5HT), histamine, acetylcholine, bradykinin and angiotensin, causes contraction of the strip while ADH has no effect. Moreover, the presence of 5HT in small amounts increases the sensitivity of the preparation towards catecholamines and abolishes the interference of the other contractor substances in the blood.

In the current work the sensitivity of the rat stomach strip towards some of the substances which might be released during haemorrhage was tested in few early experiments. Our findings confirmed those of Vane and his co-workers and showed that acetylcholine, angiotensin, histamine and 5HT cause contraction of the strip preparation, while adrenaline and noradrenaline caused relaxation. Unfortunately, bradykinin and ADH were not tested in this study.